The Applicants also request that the enclosed substitute Sequence Listing be entered into the application. The enclosed Sequence Listing differs from that filed on February 5, 2002 only in that the pages are numbered. No new matter is included. This paper is also accompanied by a Statement pursuant to 37 CFR §1.821 which states that the computer readable copy of the sequence listing and the paper copy are the same.

As a convenience to the Examiner, the following amendments to the specification incorporate the amendments requested in the Preliminary Amendment of November 3, 2000.

A copy of the sequence listing as submitted to the U.S. Patent & Trademark Office on February 5, 2002 is also enclosed.

Amendments

In the Specification:

Please enter the enclosed substitute sequence listing.

On page 1, line 3, after the title, insert the following paragraph:

–This is a contiunuation of U.S. Application Serial No. 08/484,893, filed June 7, 1995 (allowed), which in turn is a continuation of U.S. Application Serial No. 07/971,857, filed January 8, 1993 (now U.S. Patent No. 5,969,108), which in turn is the U.S. national phase of PCT/GB91/01134, filed 10 July 1991.- -

On page 35, between lines 12 and 13, insert the following subtitle

- - Summary of the Invention - -

Replace the paragraph bridging pages 42 and 43 with the following rewritten paragraph:

- - The rgdp may be a bacteriophage, the host a bacterium, and said component of the rgdp a capsid protein for the bacteriophage. The phage may be a filamentous phage. The phage may be selected from the class I phages fd, M13, f1, If1, lke, ZJ/Z, Ff and the class II phages Xf, Pf1 and Pf3. The phage may be fd or a derivative of fd. The derivative may be tetracycline resistant. The said sbp member or polypeptide chain thereof may be expressed as a fusion with the gene III capsid protein of phage fd or its counterpart in another filamentous phage. The sbp member or polypeptide chain thereof may be inserted in the N-terminal region of the mature capsid protein downstream of

a secretory leader peptide. The sequence may be inserted after amino acid +1 of the mature protein. The site for insertion may be flanked by short sequences corresponding to sequences which occur at each end of the nucleic acid to be inserted. For example where 4 the protein domain is an immunoglobulin domain, the insertion site in the phage may be flanked by nucleotide sequences which code for the first five amino acids and the last five amino acids of the Ig domain. Such flanking nucleotide sequences are shown in figure 4(2) B and C, wherein the site-flanking nucleotide sequences encode amino acid sequences QVQLQ (SEQ ID NO:1) and VTVSS (SEQ ID NO:2) which occur at either end of the VH domain, or QVQLQ (SEQ ID NO:1) and LEIKR (SEQ ID NO:3) which occur at either end of the Fv (combined VH + VL) domain. Each of these sequences flanking the insertion site may include a suitable cleavage site, as shown in Figure 4(i) and Figure 4(ii). - -

Please replace the paragraph starting at page 58, line 4, with the following rewritten paragraph:

--The applicants have also devised a series of novel selection techniques that are practicable only because of the unique properties of rgdps. The general outline of some screening procedures is illustrated in figure 2(i) and figure 2(ii) using pAbs as an example type of rgdp.--

Please replace the paragraph starting at page 58, line 10, with the following rewritten paragraph:

--The population/library of pAbs to be screened could be generated from immunised or other animals; or be created in vitro by mutagenising pre-existing phage antibodies (using techniques well-known in the art such as oligonucleotide directed mutagenesis (Sambrook, J., *et al.*, 1989 Molecular Cloning a Laboratory Manual, Cold Spring Harbor Laboratory Press). This population can be screened in one or more of the formats described below with reference to figure 2(i) and figure 2(ii), to derive those individual pAbs whose antigen binding properties are different from sample c.--

On page 65, immediately following line 20, please insert the following title:

- - Brief Description of the Drawings- -

On page 65, lines 23-29, replace the paragraph with the following rewritten paragraph:

- - Figures 2(i) and 2(ii) show schematically selection techniques which utilise the unique properties of pAbs; 2(i) shows a binding/elution system; and (2ii) shows a competition system (p=pAb; ag=antigen to which binding by pAb is required; c=competitor population e.g. antibody, pAb, ligand; s=substrate (e.g. plastic beads etc); d=detection system.- -

On page 66, lines 5-23, replace the paragraph with the following rewritten paragraph:

- - Figures 4(i)-4(ii) show the nucleotide sequences for the oligonucleotides and vectors. All sequences are drawn 5' to 3' and are numbered according to Beck et al., 1978, Nucl. Acid Res., 5: 4495-4503. 4(i) shows the sequences of the oligonucleotides used for mutagenesis (oligo's 1 and 2) or sequencing (oligo 3). The sequences shown were synthesized on an Applied Biosystems, oligonucleotide synthesizer and are complementary to the single stranded form of fd-tet (they are in the anti-sense form with respect to gene III). 4(ii) shows the sequences of the various constructs around the gene III insertion site. These sequences are drawn in the sense orientation with respect to gene III; (A) fd-tet (and fdTδBst) (B) fdTPs/Bs and (C) fdTPs/Xh. The key restriction enzyme sites are shown along with the immunoglobulin amino acids contributed by the vectors, (amino acid single letter code is used, see Harlow, E., and Lane, D., 1988 supra.).- -

On page 68, lines 8-14, please replace the paragraph with the following rewritten paragraph:

- - Figures 12a-12b show oligonucleotide probing of affinity purified phage. 10^{12} phage in the ratio of 1 pAb (D1.3) in 4 x 10^4 fdTPS/Bs phages were affinity purified and probed with an oligonucleotide specific for pAb (D1.3) A is a filter after one round of affinity purification (900 colonies total) and B is a filter after two rounds (372 colonies total).- -

On page 69, lines 1-5, replace the paragraph with the following rewritten paragraph:

- -Figure 16(i) shows the structure of gene III and the native BamHI site into which a scFv coding sequence was inserted in example 13 and figure 16(ii) shows the natural peptide linker sites A and B for possible insertion of scFv coding sequences.- -

On page 70, lines 11-14, replace the paragraph with the following rewritten paragraph:

- - Figures 23(i)-Fig. 23(ii) show the digestion pattern seen when individual clones, selected at random from a library of single chain Fv antibody genes derived from an immunised mouse; are digested with BstN1.- -

On page 70, lines 23-25, please replace the paragraph with the following rewritten paragraph:

- - Fig. 26A shows the phagemid pHEN1 a derivative of pUC119 described in example 24; and the cloning sites in the phagemid pHEN.- -

On page 72, lines 16-24, please replace the paragraph with the following rewritten paragraph:

- - Figures 35A-35B are Western Blots showing ultrafiltration of phage-enzyme 100µl of 50 fold concentrate of phage (representing 5mls of culture supernatant) was centrifuged through ultrafiltration membranes with nominal molecular weight retention of 300,000 daltons. Western blots of flow through and retentate fractions were detected with anti-alkaline phosphatase antiserum. The equivalent of 800µl of original culture supernatant was run on the gel.- -

On page 72, lines 25-28, please replace the paragraph with the following rewritten paragraph:

- - Fig. 35A. Phage were grown in TG1 cells. a) fd-phoAla166 before ultrafiltration (short exposure). b) fd-phoAla166 before ultrafiltration. c) fd-phoAla166 material retained on ultrafiltration membrane.- -

On page 73, lines 1-6, please replace the paragraph with the following rewritten paragraph:

- - Fig. 35B. Phage were grown in KS272 cells. a) fd-phoAla166 before ultrafiltration. b) fd-phoAla166 material retained on ultrafiltration membrane. c) fdCAT2. d) fdCAT2 mixed with purified alkaline phosphatase before ultrafiltration. e) Retentate from sample d. f) Flow through from sample d.- -

On page 74, lines 19-21, please replace the paragraph with the following rewritten paragraph:

- - Figures 38A-38B. Western blot of PEG precipitated phage used in ELISA probed with anti-g3p. Free g3p and the g3p-scFvD1.3 fusion bands are arrowed. - -

On page 75, lines 8-15, please replace the paragraph with the following rewritten paragraph:

- - Fig. 38A samples contain the equivalent of 8µl of phagemid culture supernatant per track, and 80µl of the fd supernatant (10-fold lower phage yield than the phagemid). Fig. 38B phagemid samples are those used in Fig. 38A at a five-fold higher sample loading (equivalent to 40µl of culture supernatant per track) to enable visualisation of the fusion band in samples rescued with parental M13K07.- -

On page 76, lines 8-9, please replace the paragraph with the following rewritten paragraph:

- - Figures 44(i)-(ii) show the DNA sequence of scFv B18 (anti-NP).- -

On page 76, lines 23-29, please replace the paragraph with the following rewritten paragraph:

- - Figure 48(i) shows a map of plasmid pJM1-FabD1.3 which is used for the expression of soluble human Fab fragments and as a template for the synthesis of linker DNA for Fab assembly. Figure 48(ii) is a schematic representation of sequences encoding a Fab construct. Fig. 48(iii) shows the sequence of DNA template for the synthesis of linker DNA for Fab assembly.- -

On page 77, lines 5-13, please replace the paragraph with the following rewritten paragraph:

- - Figures 50(i)-(ii). ELISA assay of phage antibodies using plates coated with turkey egg lysogyme. Two clones B1 and A4 are shown derived by mutagenesis and selection from pAbD1.3 (example 45). Concentration (x axis) refers to the concentration of phage for each sample relative to the concentration in culture supernatant. B1 has raised binding to turkey egg lysogyme compared to D1.3. A4 has reduced binding to hen egg lysogyme compared to D1.3.- -

On page 94, lines 12-23, please replace the paragraph with the following rewritten paragraph:

- - In vitro mutagenesis of fdTδBst was used to generate vectors having appropriate restriction sites that facilitate cloning of antibody fragments downstream of the gene III signal peptide and in frame with the gene III coding sequence. The oligonucleotide directed mutagenesis system version 2 (Amersham International) was used with oligo 1 (figure 4(i)) to create fdTPs/Bs (to facilitate cloning of VH fragments). The sequence offdTPs/Bs (figure 4(i)) was confirmed using the sequenase version 2.0 kit (USB Corp., PO Box 22400, Cleveland, Ohio, 44122, UsA.) with oligo 3 (figure 4) as a primer.- -

On page 102, lines 1-12, please replace the paragraph with the following rewritten paragraph:

- - The oligonucleotide:

5'ACT TTC AAC AGT TTC TGC GGC CGC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG TGC ACT GTG AGA ATA GAA 3' (SEQ ID NO:4) was synthesised (supra fig 4 legend) and used to mutagenise fdTPs/Xh using an in vitro mutagenesis kit from Amersham International as described in example 1, to create fd-CAT2. The sequence of fd-CAT2 was checked around the site of manipulation by DNA sequencing. The final sequence around the insertion point within gene III is shown in figure 8. N.B. fdCAT2 is also referred to herein by the alternative terminologies fd-tet-DOG1 and fdDOG1.- -

In the paragraph bridging pages 105-106, please replace the paragraph with the following rewritten paragraph:

- - The starting point for this example was the clone Fab D1.3 in pUC19, a map of which is shown in figure 10. The regions hybridising with the oligonucleotides KSJ6 and 7 below are shown underlined in fig 10. The sequence encoding the VH-CH1 region (defined at the 5' and 3' edges by the oligonucleotides KSJ6 and 7 below) was PCR amplified from Fab D1.3 in pUC19 using oligonucleotides KSJ 6 and 7, which retain the Pst I site at the 5' end and introduce a Xho I site at the 3' end, to facilitate cloning into fd CAT2. The sequences for the oligonucleotides KSJ6 and 7 are shown below. The underlined region of KSJ7 shows the portion hybridising with the sequence for D1.3. KSJ6:5' AGG TGC AGC TGC AGG AGT CAG G 3' (SEQ ID NO:5)
KSJ7: 5' GGT GAC CTC GAG <u>TGA AGA TTT GGG CTC AAC TTT C</u> 3' (SEQ ID NO:6)- -

Please replace the paragraph bridging pages 108 and 109, with the following rewritten paragraph:

- -The applicant purified pAb (D1.3) (originally called fdTscFvD1.3 in example 2) from mixtures using antigen affinity columns. pAb (D1.3) was mixed with vector fd phage (see table 1) and approximately 10^{12} phage passed over a column of lysozyme-Sepharose (prepared from cyanogen bromide activated sepharose 4B (Pharmacia, Milton Keynes, Bucks, UK.) according to the manufacturers instructions. TG1 cells were infected with appropriate dilutions of the elutes and the colonies derived, were analysed by probing with an oligonucleotide that detects only the pAb (D1.3) see Table 1 and Figs. 12A and 12B. A thousand fold enrichment of pAb(D1.3) was seen with a

single column pass. By growing the enriched phage and passing it down the column again, enrichments of up to a million fold were seen. - -

On page 109, lines 15-26, please replace the paragraph with the following rewritten paragraph:

- - Enrichment was also demonstrated using purely immunological criteria. For example, 10^{12} phage (at a ratio of 1 pAb (D1.3) to 4 x 10^6 fdTPs/Bs) was subjected to two rounds of affinity selection, and then 26 colonies picked and grown overnight. The phage was then assayed for lysozyme binding by ELISA (as example 6). Five colonies yielded phage with lysozyme binding activities, see table 1, and these were shown to encode the scFv (D1.3) by PCR screening (example 13, using 30 cycles of 1 minute at 92°C, 1 minute at 60°C, 1 minute at 72°C using CDR3PCR1 and oligo 3 (Fig. 4(i)) as primers).- -

On page 110, lines 4-24, please replace the paragraph with the following rewritten paragraph:

- - Approximately 10^{12} phage particles in 1ml MPBS were loaded onto a 1 ml lysozyme-Sepharose affinity column which had been prewashed in MPBS. The column was washed in turn with 10 ml PBS; then 10 ml 50 mM Tris-HCl, 500 mM Nacl pH 7.5; then 10ml 50 mM Tris-HCl 500 mM NaCl pH 8.5; then 5 mls 50 mM Tris-HCl, 500 mM NaCl pH 9.5 (adjusted with triethylamine) and then eluted with 5 ml 100 mM triethylamine. The eluate was neutralised with 0.5 M sodium phosphate buffer pH 6.8 and the phage plated for analysis. For a second round of affinity chromatography, the first column eluate was plated to about 30,000 colonies per petri dish. After overnight growth, colonies were then scraped into 5 ml 2 x TY medium, and a 20 µl aliquot diluted into 10 ml fresh medium and grown overnight. The phage was PEG precipitated as described above, resuspended in 1 ml MPBS and loaded onto the column, washed and eluted as above.
Oligonucleotides sythesised:
CDR3PCR1 5'TGA GGA C(A or T) C(A or T) GC CGT CTA CTA CTG TGC 3' (SEQ ID NO:7)- -

On page 113, lines 8-22, please replace the paragraph with following rewritten paragraph:

- - 3 x 10^{10} phage in 10 mls of PBSM at the ratios of pAb D1.3 to pAb NQ11 shown in table 2 were passed over a 1 ml lysozyme Sepharose column. Washing, elution and other methods were as described in example 8 unless otherwise stated. Eluates from the columns were used to infect

TG1 cells which were then plated out. Colonies were probed with a probe which distinguishes pAb D1.3 from pAb NQ11. The sequence of this oligonucleotide (D1.3CDR3A) is:-
5'GTA GTC AAG CCT ATA ATC TCT CTC 3' (SEQ ID NO:8). Table 2 presents the data from this experiment. An enrichment of almost 1000 fold was achieved in one round and an enrichment of over a million fold in two rounds of purification. This parallels the result described in example 8.- -

Replace the paragraph bridging pages 113-114, with the following rewritten paragraph:

- - As an example of the expression of a functional enzyme on the bacteriophage surface, the applicants have chosen bacterial alkaline phosphatase, an enzyme that normally functions as a dimer (McCracken, S. and Meighen, E., J. Biol. Chem. 255, p2396-2404, (1980)). The oligonucleotides were designed to generate a PCR product with an Apa L1 site at the 5' end of phoA gene and a Not 1 site at its 3' end, thus facilitating cloning into fd-CAT 2 to create a gene III fusion protein. The oligonucleotides synthesised were: phoA1:5' TAT TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG 3' (SEQ ID NO:9) and, phoA2:5' ACA TGT ACA TGC GGC CGC TTT CAG CCC CAG AGC GGC TTT C 3' (SEQ ID NO:10). The sequence of the phoA gene is presented in Chang C. N. et al., Gene 44, p121-125 (1986). The plasmid amplified (pEK86) contains an alkaline phosphate gene which differs from the sequence of Chang et al, by a mutation which converts arginine to alamine at position 166.- -

Please replace the paragraph on page 118, lines 7-21, with the following rewritten paragraph:

- - DNA fragments encoding scFv's from D1.3 or NQ11 were generated by PCR using the primers shown below. These primers were designed to generate a fragment with BamH1 sites near both the terminii, to enable cloning into the BamH1 site of gene3 (see figure 16(i)). The oligonucleotides used, also ensure that the resulting PCR product lacks Pst1 and Xhol restriction sites normally used for manipulating the scFv's (see figure 16(1)). This will facilitate subsequent manipulation of a second antibody fragment in the usual way at the N terminus of gene 3. The oligonucleotides used were:-
G3Baml 5'TTT AAT GAG GAT CCA CAG GTG CAG CTG CAA GAG 3' (SEQ ID NO:11)
G3Bam2 5'AAC GAA TGG ATC CCG TTT GAT CTC AAG CTT 3' (SEQ ID NO:12).- -

Please replace the paragraph on page 120, lines 1-14, with the following rewritten paragraph:

- - It may be possible to clone into alternative sites to retain binding activity. The peptide repeats present in gene III may provide such a site (figure 16 blocks A and B). This can be done by inserting a BamHl site and using the PCR product described above. To facilitate this, the natural BamHl site was removed by mutagenesis with the oligonucleotide G3mutδBam shown below (using an in vitro mutagenesis kit (Amersham International)):-

G3mutδBam 5' CA AAC GAA TGG GTC CTC CTC ATT A 3' (SEQ ID NO:13)

The underlined residue replaces an A residue, thereby removing the BamHl site. DNA was prepared from a number of clones and several mutants lacking BamHl sites identified by restriction digestion.- -

Please replace the paragraph on page 120, lines 15-22, with the following rewritten paragraph:

- -The oligonucleotide G3 Bamlink was designed to introduce a BamHl site at a number of possible sites within the peptide linker sites A and B, see figure 16(ii). The sequence of the linker is: Bamlink 5'CC (G or A) CC ACC CTC GGA TCC (G or A) CC ACC CTC 3' (SEQ ID NO:14). Its relationship to the peptide repeats in gene III is shown in figure 16.- -

Replace the paragraph on page 121, lines 1-16 with the following paragraph:

- - 1. cDNA is prepared from spleen RNA from an appropriate mouse and the VH and VLK repertories individually amplified. Separately, primers reverse and complementary to VH1FOR-2 (domain 1) and VLK2BACK (domain 2) are used to amplify an existing scFv-containing DNA by PCR. (The term FOR refers to e.g. a primer for amplification of sequences on the sense strand resulting in antisense coding sequences. The term BACK refers to e.g. a primer for amplification of sequences on the antisense strand resulting in sense coding sequences). This generates a 'linker' molecule encoding the linker with the amino acid sequence (1 letter code) $(GGGGS)_3$ (SEQ ID NO:15) which overlaps the two primary (VH and VLK) PCR products. - -

Please replace the paragraph on page 126, lines 14-21, with the following rewritten paragraph:

- - The primers anneal to the 3' end. Examples of kappa light chain primers are MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (provided under 'Primer Sequences' below) and examples of heavy chain primers are MIGG1, 2 (CTG GAC AGG GAT CCA GAG TTC CA) (SEQ ID NO:16) and MIGG3 (CTG GAC AGG GCT CCA TAG TTC CA) (SEQ ID NO:17) which anneal to CH1.- -

Please replace the paragraph beginning at page 127, line 28, with the following rewritten paragraph:

--Purify on a 2% 1mp (low melting point agarose/TAE (tris-acetate EDTA)gel and extract the DNA to 20 µl H_2O per original PCR using a GENECLEAN kit (see earlier; Bio101, La Jolla CA, USA) in accordance with the manufacturers instructions.--

Please replace the paragraph bridging page 132 (starting at line 10) through page 133 (line 8) with the following:

- - Primer sequences

Primary PCR oligos (restrictions sites underlined):

VH1FOR-2 TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC (SEQ ID NO:18)

VH1BACK AGG TSM ARC TGC AGS AGT CWG G (SEQ ID NO:19)

MJK1FONX CCG TTT GAT TTC CAG CTT GGT GCC (SEQ ID NO:20)

MJK2FONX CCG TTT TAT TTC CAG CTT GGT CCC (SEQ ID NO:21)

MJK4FONX CCG TTT TAT TTC CAA CTT TGT CCC (SEQ ID NO: 22)

MJK5FONX CCG TTT CAG CTC CAG CTT GGT CCC (SEQ ID NO:23)

VK2BACK GAC ATT GAG CTC ACC CAG TCT CCA (SEQ ID NO:24)

Ambiguity codes M = A or C, R = A or G, S = G or C,

W = A or T

PCR oligos to make linker:

LINKFOR TGG AGA CTC GGT GAG CTC AAT GTC (SEQ ID NO:25)

LINKBACK GGG ACC ACG GTC ACC GTC TCC TCA (SEQ ID NO:26)

For adding restriction sites:

HBKAPA10 CAT GAC CAC AGT GCA CAG GTS MAR CTG CAG SAG TCW GG (SEQ ID NO:27)

JKINOT10 GAG TCA TTC TGC GGC CGC CCG TTT GAT TTC CAG CTT GGT GCC (SEQ ID NO:28)

JK2NOT10 GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAG CTT GGT CCC (SEQ ID NO:29)

JK4NOT10 GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAA CTT TGT CCC (SEQ ID NO:30)

JK5NOT10 GAG TCA TTC TGC GGC CGC CCG TTT CAG CTC CAG CTT GGT CCC (SEQ ID NO:31)- -

On page 134, please replace lines 7-11, with the following rewritten paragraph:

- - RPDGF3 5' CAC AGT GCA CTG GTC GTC ACA CCC CCG GGG CCA GAG CTT GTC CTC AAT GTC TCC AGC ACC TTC GTT CTG 3' (SEQ ID NO:32)

RPDGF2 5' GAT CTC GAG CTT AAA GGG CAA GGA GTG TGG CAC 3' (SEQ ID NO:33)- -

On page 139, please replace lines 1-7, with the following rewritten paragraph:

- -Gene III from fd-CAT2 (example 5) and the gene III scFv fusion from fd-CAT2 scFvD1.3 was PCR-amplified using the primers A and B shown below:

Primer A: TGC GAA GCT TTG GAG CCT TTT TTT TTG GAG ATT TTC AAC G (SEQ ID NO:34)

Primer B: CAG TGA ATT CCT ATT AAG ACT CCT TAT TAC GCA GTA TGT TAG C (SEQ ID NO:35)- -

On Page 145, please replace lines 1-26 with the following rewritten paragraph:

- - Vector fdCAT2 was extensively digested with Not1 and ApaL1., purified by electroelution (Sambrook et al.a989 supra) and 1 μg ligated to 0.5 μg (5 μg for the hierarchial libraries: see example 22) of the assembled scFv genes in 1 ml with 8000 units T4 DNA ligase (New England Biolabs). The ligation was carried out overnight at 16°C. Purified ligation mix was electroporated

in six aliquots into MC1061 cells (W. J. Dower, J. F. Miller & C. W. Ragsdale Nucleic Acids Res. 16 6127-6145 1988) and plated on NZY medium (Sambrook et al. 1989 supra) with 15μg/ml tetracycline, in 243x243 mm dishes (Nunc): 90-95% of clones contained scFv genes by PCR screening. Recombinant colonies were screened by PCR (conditions as in example 7 using primers VH1BACK and MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (see example 14) followed by digestion with the frequent cutting enzyme BstN1 (New England Biolabs, used according to the manufacturers instructions). The library of $2x10^5$ clones appeared diverse as judged by the variety of digestion patterns seen in Figure 23(i) and Figure 23(ii), and sequencing revealed the presence of most VH groups (R. Dildrop, Immunol. Today 5 85-86. 1984) and VK subgroups (Kabat. E.A. et al. 1987 supra) (data not shown). None of the 568 clones tested bound to phOx as detected by ELISA as in example 9.- -

Please replace the paragraph bridging page 147 (starting at line 27) through page 148 (line 11) with the following rewritten paragraph:

- - To sequence clones, template DNA was prepared from the supernatants of 10 ml cultures grown for 24 hours, and sequenced using the dideoxy method and a Sequenase kit (USB), with primer LINKFOR (see example 14) for the VH genes and primer fdSEQ1 (5'-GAA TTT TCT GTA TGA GG -3') (SEQ ID NO:36) for the Vk genes. Twenty-three of these hapten-binding clones were sequenced and eight different VH genes (A to H) were found in a variety of pairings with seven different Vk genes (a to g) (Fig. 24). Most of the domains, such as VH-B and Vk-d were 'promiscuous', able to bind hapten with any of several partners.- -

Please replace the paragraph bridging page 148 (starting at line 10) through page 149 (line 9) with the following rewritten paragraph:

- -The sequences of the V-genes were related to those seen in the secondary response to phOx, but with differences (Fig. 24). Thus phOx hybridomas from the secondary response employ somatically mutated derivatives of three types of Vk genes - Vkoxl. 'Vkox-like' and Vk45.1 genes (C. Berek, G. M. Griffiths & C. Milstein Nature 316 412-418 (1985). These can pair with VH genes from several groups, from Vkoxl more commonly pairs with the VHoxl gene (VH group 2. R.Dildrop uupra). Vkoxl genes are always, and Vkox-like genes often, found in association with heavy chains (including VHoxl) and contain a short five residue CDR3, with the sequence motif

Asp-X-Gly-X-X (SEQ ID NO:37) in which the central glycine is needed to create a cavity for phOx. In the random combinatorial library however, nearly all of the VH genes belonged to group 1, and most of the Vk genes were ox-like and associated with VH domains with a five residue CDR3, motif Asp/Asn-X-Gly-X-X (SEQ ID NO:38) (Fig. 24). Vkoxl and VHoxl were found only once (Vk-f and VH-E), and not in combination with each other. Indeed Vk-f lacks the Trp91 involved in phOx binding and was paired with a VH (VH-C) with a six residue CDR3.- -

Please replace the paragraph bridging page 151 (starting at line 9) through page 152 (line 8) with the following rewritten paragraph:

- -The promiscuity of the VH-B and Vk-d domains prompted the applicants to force further pairings, by assembling these genes with the entire repertoires if either Vk or VH genes from the same immunised mice. The resulting 'hierarchical' libraries, (VH-B x Vk-rep and VH-rep x Vk-d), each with $4x10^7$ members, were subjected to a round of selection and hapten-binding clones isolated (Table 4). As shown by ELISA, most were strong binders. By sequencing twenty-four clones from each library, the applicants identified fourteen new partners for VH-B and thirteen for Vk-d (Fig. 24). Apart from VH-B and Vk-c, none of the previous partners (or indeed other clones) from the random combinatorial library was isolated again. Again the Vk genes were mainly ox-like and the VH genes mainly group 1 (as defined in Dildrop, R. 1984 supra), but the only examples of Vkoxl (Vk-h, -p, -q and -r) have Trp91, and the VH-CDR3 motif Asp-X-Gly-X-X (SEQ ID NO:37) now predominates. Thus some features of the phOx hybridomas seemed to emerge more strongly in the hierarchial library. The new partners differed from each other mainly by small alterations in the CDRs, indicating that much of the subtle diversity had remained untapped by the random combinatorial approach. More generally it has been shown that a spectrum of related antibodies can be made by keeping one of the partners fixed and varying the other, and this could prove invaluable for fine tuning of antibody affinity and specificity.- -

Please replace the paragraph bridging page 153 (starting at line 8) through page 154 (line 26) with the following rewritten paragraph:

- - Clones VH-B/Vk-b and VH-B/Vk-d were reamplified with MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (see example 14) and VH1BACK-Sfil (5'-TCG CGG CCC AGC CGG CCA TGG CC(G/C) AGG T(C/G)(A/C) A(A/G)C TGC AG(C/G) AGT C(A/T)G G-3') (SEQ

ID NO:39), a primer that introduces an SfiI site (underlined) at the 5' end of the VH gene. VH-B/Vk-d was cloned into a phagemid e.g. pJM1 (a gift from A. Griffiths and J. Marks) as an SfiI-NotI cassette, downstream of the pelB leader for periplasmic secretion (M. Better at al. supra), with a C-terminal peptide tag for detection (see example 24 and figure), and under th control of a P_L promoter (H. Shimatake & M. Rosenberg Nature 292 128-132 1981). The phagemid should have the following features: a) unique SfiI and Not1 restriction sites downstream of a pelB leader; b) a sequence encoding a C-terminal peptide tag for detection; and c) a λ P_L promoter controlling expression. 10 litre cultures of E.coli N4830-1 (M. E. Gottesman, S. Adhya & A. Das J.Mol.Biol 140 57-75 1980) harbouring each phagemid were induced as in K. Nagai & H. C. Thogerson (Methods Enzymol 153 461-481 1987) and supernatants precipitated with 50% ammonium sulphate. The resuspended precipitate was dialysed into PBS + 0.2 mM EDTA (PBSE), loaded onto a 1.5ml column of phOx:Sepharose and the column washed sequentially with 100 ml PBS: 100 ml 0.1 M Tris-HC1, 0.5 M NaC1, pH 8.0: 10ml 50 mM citrate, pH 5.0: 10 ml 50 mM citrate, pH4.0, and 20 ml 50 mM glycine, pH 3.0. scFv fragments were eluted with 50 mM glycine, pH 2.0, neutralised with Tris base and dialysed against PBSE. VH-B/Vk-b was cloned into a phagemid vector based on pUC119 encoding identical signal and tag sequences to pJM1, and expression induced at 30°C in a 10 litre culture of E.coli TG1 harbouring the phagemid as in D. de Bellis & I. Schwartz (1980 Nucleic Acids Res 18 1311). The low affinity of clone VH-B/Vk-b made its purification on phOx-Sepharose impossible. Therefore after concentration by ultrafiltration (Filtron, Flowgen), the supernatant (100 ml of 600 ml) was loaded onto a 1 ml column of protein A-Sepharose cpoupled (E. Harlow & D. Lane 1988 supra) to the monoclonal antibody 9E10 (Evan, G. I. et al. Mol.Cell Biol.5 3610-3616 1985) that recognises the peptide tag. The column was washed with 200 ml PBS and 50 ml PBS made 0.5 M in NaC1. scFv fragments were eluted with 100 ml 0.2M glycine, pH 3.0, with neutralisation and dialysis as before.- -

Please replace the paragraph bridging page 154 (starting at line 27) through page 155 (line 28) with the following rewritten paragraph:

- - The Kd ($1.0 \pm 0.2 \times 10^{-8}$ M) for clone VH-B/Vk-d was determined by fluorescence quench titration with 4-E-amino-butyric acid methylene 2-phenyl-oxazol-5-one (phOx-GABA Co. Makela et al, 1978 supra). Excitation was at 280 nm, emission was monitored at 340 nm and the K_d calculated. The K_d of the low affinity clone VH-B/Vk-b was determined as $1.8 \pm 0.3 \times 10^{-5}$ M (not

shown). To minimise light adsorption by the higher concentrations of phOx-GABA required, excitation was at 260 nm and emission was monitored at 304 nm. In addition the fluorescence values were divided by those from a parallel titration of the lysozyme binding Fv fragment D1.3. The value was calculated as in H. N. Eisen Meth.Med.Res. 10 115-121 1964. A mixture of clones VH-B/Vk-b and VH-B/Vk-d, $7x10^{10}$ TU phage in the ratio 20 VH-B/Vk-b : 1 VH-B/Vk-d were loaded onto a phOx-BSA-Sepharose column in 10 ml MPBS and eluted as above. Eluted phage were used to reinfect E.coli TG1, and phage produced and harvested as before. Approximately 10^{11} TU phage were loaded onto a second affinity column and the process repeated to give a total of three column passes. Dilutions of eluted phage at each stage were plated in duplicate and probed separately with oligonucleotides specific for Vk-b (5'-GAG CGG GTA ACC ACT GTA CT-3')(SEQ ID NO:40) or Vk-d (5'-GAA TGG TAT AGT ACT ACC CT-3')(SEQ ID NO:41). After these two rounds, essentially all the eluted phage were VH-B/Vk-d (table 4). Therefore phage antibodies can be selected on the basis of the antigen affinity of the antibody displayed.

Example 24 - -

On page 156, please replace lines 4-21 with the following rewritten paragraph:

- - The phagemid pHEN1 (figure 26(a)) is a derivative of pUC119 (Vieira, J. & Messing, J. Methods Enzymol 153 pp 3-11, 1987). The coding region of g3p from fdCAT2, including signal peptide and cloning sites, was amplified by PCR, using primers G3FUFO and G3FUBA (given below) (which contain EcoRI and HindIII sites respectively), and cloned as a HindIII-EcoRI fragment into pUC119. The HindIII-NotI fragment encoding the g3p signal sequence was the replaced by a pelB signal peptide (Better, M. et al. Science 240 1041-1043, 1988) with an internal SfiI site, allowing antibody genes to be cloned as fiI-NotI fragments. A peptide tag, c-myc, (Munro, S. & Pelham, H. Cell 46 291-300, 1986) was introduced directly after the NotI site by cloning an oligonucleotide cassette, and followed by an amber codon introduced by site-directed mutagenesis using an in vitro mutagenesis kit (Amersham International) (figure 26(b)).- -

On page 156, please replace lines 23 through 27 with the following rewritten paragraph:

- - G3FUFO,5'-CAG TGA ATT CTT ATT AAG ACT CCT TAT TAC GCA GTA TGT TAG C-3' (SEQ ID NO:42);

G3FUBA,5'-TGC GAA GCT TTG GAG CCT TTT TTT TTG GAG ATT TTC AAC G-3' (SEQ ID NO:43);

Example 25 - -

On page 158, please replace lines 12 through 28 with the following rewritten paragraph:

- - VH1BACKAPA, 5'-CAT GAC CAC AGT GCA CAG GT(C/G) (A/C)A(A/G) CTG CAG (C/G)AG TC(A/T) GG-3' (SEQ ID NO:44);

VH1BACKSFI15, 5'-CAT GCC ATG ACT CGC GGC CCA GCC GGC CAT GGC C(C/G)A GGT (C/G)(A/C)A (A/G)CT GCA G(C/G)A GTC (A/T)GG-3' (SEQ ID NO:45);

FABNOTFOH, 5'-CCA CGA TTC TGC GGC CGC TGA AGA TTT GGG CTC AAC TTT CTT GTC GAC-3' (SEQ ID NO:46);

FABNOTFOK, 5'-CCA CGA TTC TGC GGC CGC TGA CTC TCC GCG GTT GAA GCT CTT TGT GAC-3' (SEQ ID NO:47);

MVKBAAPA, 5'-CAC AGT GCA CTC GAC ATT GAG CTC ACC CAG TCT CCA-3' (SEQ ID NO:48);

MVKBASFI, 5'-CAT GAC CAC GCG GCC CAG CCG GCC ATG GCC GAC ATT GAG CTC ACC CAG TCT CCA-3' (SEQ ID NO:49);

VK3F2NOT, 5'-TTC TGC GGC CGC CCG TTT CAG CTC GAG CTT GGT CCC -3' (SEQ ID NO:50).

Restriction sites are underlined.

Rescue of Phage and Phagemid particles - -

Please replace the paragraph bridging page 160 (starting at line 26) through page 161 (line 7) with the following rewritten paragraph:

- - The phagemid vector, pHEN1 (fig. 26(a)), is based upon pUC119 and contains restriction sites (SfiI and NotI) for cloning the fusion proteins. Here the transcription of antibody-g3p fusions is driven from the inducible lacZ promoter and the fusion protein targetted to the periplasm by means of the pelB leader. Phagemid was rescued with VCSM13 helper phage in 2xTY medium containing no glucose or IPTG: under these conditions there is sufficient expression of antibody-g3p. Fab and scFv fragments of NQ10.12.5 cloned into pHEN1 for display were shown to bind to phOx-BSA (but not BSA) by ELISA (Table 5) using the same criterion as above.- -

On page 179, please replace lines 10-15 with the following rewritten paragraph:

- -The construct fdphoAla166 (derived in example 11) was converted back to the wild type residue (arginine) at position 166 by in vitro mutagenesis (Amersham International) using the printer APARG166:5' TAGCATTTGCGCGAGGTCACA 3' (SEQ ID NO:51).
This construct with the wild type insert was called fdphoArg166.

On page 183, please replace lines 1-17, with the following rewritten paragraph:

- - Phage-enzyme or free alkaline phosphatase (83ng) mixed with vector phage were passed through filters with a nominal molecular weight limit of 300,000 daltons (Ultrafree-MC filters, Millipore). Figure 35 A again shows that the band of Mr, 115,000 is the major product reactive with anti-BAP antiserum. This and the other minor products reactive with anti-BAP are present in material retained by the ultrafiltration membrane. Analysis of retained and flow through fractions of phage preparations derived from KS272 demonstrates that different molecular species are being separated by the ultrafiltration membranes. Figure 35B shows the protein of Mr 115,000 is retained by the filter whereas the putative degradation products of Mr 95,000 and 60,000 found in phage preparations derived from KS272 cells, are not retained.- -

Please replace the paragraph bridging page 183 (starting at line 18) through page 184 (line 3) with the following rewritten paragraph:

- - In mixture of alkaline phosphatase and vector phage Figure 35B(c-f), free alkaline phosphatase (dimer size of 94,000 daltons) is detected in the flow through as a monomer band with Mr 47,000 on denaturing polyacrylamide gels (figure 35B), while the cross reactive molecule found in vector phage preparations (Mr 45,000) is in retained on the filter (figure 35B). This suggests that the cross reactive molecule is part of the phage particle and underlines the fact that the ultrafiltration membranes are effecting a separation. Thus the expected fusion band in this phage-enzyme is present in material retained on ultrafiltration membranes demonstrating that it is part of a larger structure as would be expected for viral bound enzyme.- -

Please replace the paragraph bridging page 184 (starting at line 13) through page 185 (line 5) with the following rewritten paragraph:

- - Chaidaroglou et al, 1988 supra have shown that substituting alanine for arginine at the active site (residue 166) leads to a reduction in the rate of catalysis. Preparations of phage displaying alkaline phosphatase with this mutation derived from TG1 and KS272 show reduced specific activities of 380 and 1400 mol substrate converted/mol phage/min respectively. Enzyme activity was measured in the retained and flow-through fractions prepared by ultrafiltration, shown in Figure 35(A) and Figure 35(B). The bulk of activity from phage-enzyme was retained on the filters whereas the majority of activity from free enzyme passes through. Therefore, the enzyme activity in these fusions behaved as would be expected for virally associated enzyme (not shown). Little or no catalytic activity is measured in preparations of vector phage from either TG1 or KS272 cells (Table 7), indication that the catalytic activities above are due to phage enzyme and not contamination with bacterial phosphatase. Addition of phage particles to soluble enzyme does not have a significant effect on activity (Table 7). - -

On page 189, please replace lines 9-15, with the following

- - VK-TERM-FOR

5' TGG AGA CTG GGT GAG CTC AAT GTC GGA GTG AGA ATA GAA AGG 3' (SEQ ID NO:52) (overlapping with VK2BACK [example 14])

and

CH1-TERM-BACK

5'AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA TCT AGC TGA TAA ACC GAT ACA ATT AAA GGC 3' (SEQ ID NO:53) (overlapping with HuIgG1-4 CH1-FOR)- -

Please replace the paragraph bridging page 191 (starting at line 28) through page 192 (line 5) with the following rewritten paragraph:

- - Aliquots of the ligation reaction were transformed into competent TG1/pUC19gIII and plated on SOB medium containing ampicillin at 100μg/ml and kanamycin at 50μg/ml. Colonies were screened for the presence of a deletion by PCR with primers gIIIFUBA and KSJ12 (CGGAATACCCAAAAGAACTGG)(SEQ ID NO:54).- -

On page 192, please replace lines 5-16, with the following rewritten paragraph:

- - KSJ 12 anneals to gene VI which is immediately downstream of gIII in the phage genome, so distinguishing gIII on the helper phage from that resident on the plasmid. Three clones gave trunctated PCR products corresponding to deletions of ca. 200, 400 and 800bp. These clones were called M13K07 gIII Δ Nos 1,2 and 3 respectively. No clones were isolated from the earlier Bal 31 time points, suggesting that these are in some way lethal to the host cell. Several clones were isolated from later time points, but none of these gave a PCR product, indicating that the deletion reaction had gone too far.- -

On page 193, please replace lines 5-18, with the following rewritten paragraph:

- - Only a minute fraction of the gIII protein on the M13K07-rescued material is present as an intact fusion (Figs. 38A-38B). The fusion protein band is induced by IPTG, so is indisputably that synthesised by the phagemid. As expected, even when the lac promoter driving gIII fusion protein synthesis is fully induced (100μM IPTG), wild type gIII protein, at a lower copy number and driven from a far weaker promoter, predominates. This is in contrast to the pattern generated by the same clone rescued with M13K07 gIIIΔNo3, and the pattern generated by fd CAT2-scFv D1.3. In both of these latter cases, there is no competition with wild-type gIII and the fusion protein band is correspondingly stronger.- -

On page 195, please replace lines 12-25 with the following:

- - OLIGONUCLEOTIDES

VHBHD13APA : 5'- CAC AGT GCA CAG GTC CAA CTG CAG GAG AGC GGT-3' (SEQ ID NO:55)

VHFHD13 : 5'- CGG TGA CGA GGC TGC CTT GAC CCC-3' (SEQ ID NO:56)

HD13BLIN : 5'- GGG GTC AGG GCA GCC TCG TCA CCG-3' (SEQ ID NO:57)

HD13FLIN3 : 5'- TGG GCT CTG GGT CAT CTG GAT GTC CGA T-3' T (SEQ ID NO:58)

VKBHD13 : 5'- GAC ATC CAG ATG ACC CAG AGC CCA-3' (SEQ ID NO:59)

VKFHD13NOT : 5'- GAG TCA TTC TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3' (SEQ ID NO:60)

MURD13SEQ : 5'- GAG GAG ATT TTC CCT GT -3' (SEQ ID NO:61)

HUMD13SEQ : 5'- TTG GAG CCT TAC CTG GC-3' (SEQ ID NO:62)

FDPCRFOR : 5'- TAG CCC CCT TAT TAG CGT TTG CCA-3' (SEQ ID NO:63)

FDPCRBAK : 5'- GCG ATG GGT GTT GTC ATT GTC GGC-3' (SEQ ID NO:64).- -

On page 199, please replace lines 20-24 with the following:

- -	TPB1	VH-HuH2-$(Gly_4\text{-}Ser)_3$-VK-HuK3	13 nM (SEQ ID NO:269)
	TPB2	VH-HuH1-$(Gly_4\text{-}Ser)_3$-VK-HuK4	180 Nm (SEQ ID NO:270)
	TPB3	VH-HuH2-$(Gly_4\text{-}Ser)_3$-VK-HuK4	(Unknown) (SEQ ID NO:271)
	TPB4	VH-HuH1-$(Gly_4\text{-}Ser)_3$-VK-HuK3	52 nM (SEQ ID NO:272) - -

Please replace the paragraph bridging page 203 (starting at line 26) through page 204 (line 18) with the following rewritten paragraph:

- -The gene for the enzyme Staphylococcal nuclease (SNase) was amplified from M13 mp18 - SNase (Neuberger, M.S. et al Nature 312 604-608, 1984) by PCR using primers with internal ApaLI (5'- GGAATTCGTGCACAGAGTGCAACTTCAACTAAAAAATTAC-3')(SEQ ID NO:65) and NotI (5'-GGGATCCGCGGCCGCTTGACCTGAATCAGCGTTGTCTTCG-3') (SEQ ID NO:66) restriction sites, cloned into phage vector fd-CAT2 after digestion with ApaLI-NotI restriction enzymes and the nucleotide sequence of the SNase gene and junctions with gene III checked by DNA sequencing. The fd-tet-SNase phage was prepared from the supernatant of infected E.coli TG1 cultures by three rounds of PEG precipitation, and the fusion protein demonstrated by SDS-gel electrophoresis and Western blotting using rabbit anti-g3p antiserum (Prof. I. Rasched, Konstanz) and peroxidase-labelled goat anti-rabbit antibodies (Sigma) (Fig.41) as described in example 27. As well as the fusion protein band (calculated Mr 59749, but runs at a higher position due to the aberrant g3p behaviour), a smaller (proteolytic ?) product is seen.- -

Please replace the paragraph bridging page 205 (starting at line 12) through page 206 (line 9) with the following rewritten paragraph:

- - The protein CD4, a member of the immunoglobulin superfamily, is a cell surface receptor involved in MHC class II restricted immune recognition. It is also recognised by the protein gp120 derived from the human immunodeficiency virus (AIDS virus). The first two domains (named V1 and V2, residues 1-178) of the surface antigen CD4 were amplified from pUC13-T4 (gift from T.

Simon) containing the human cDNA of CD4, by PCR using primers with internal ApaLI (5'-GGA ATT CGT GCA CAG AAG AAA GTG GTG CTG GGC AAA AAA GGG G-3') (SEQ ID NO:67) and NotI (5'-GGG ATC CGC GGC CGC AGC TAG CAC CAC GAT GTC TAT TTT GAA CTC-3') (SEQ ID NO:68) restriction sites. After digestion with these two enzymes, the PCR-product was cloned into fdCAT2, and the complete nucleotide sequence of the CD4-V1V2 DNA and junctions with gene III checked by dideoxy sequencing using oligonucleotides fd-seq1 (5'-GAA TTT TCT GTA TGA GG)(SEQ ID NO:69), CD4-seq1 (5'-GAA GTT TCC TTG GTC CC-3') (SEQ ID NO:70) and CD4-seq2 (5'-ACT ACC AGG GGG GCT CT-3')(SEQ ID NO:71). In the same way, a fd-CD4-V1 version was made, linking residues 1-107 to the N-terminus of gene III, using previously mentioned primers and oligonucleotide 5'-GGG ATC CGC GGC CGC GGT GTC AGA GTT GGC AGT CAA TCC GAA CAC-3' (SEQ ID NO:72) for amplification, PCR conditions and cloning were essentially as described in example 15 except that digestion was with ApaLI and NotI (used according to the manufacturers instructions).- -

Please replace the paragraph bridging page 211 (starting at line 15) through page 212 (line 5) with the following rewritten paragraph:

- - After 4 rounds of mutation and selection, isolated clones were screened and in one or two rare examples strongly positive ELISA signals were obtained from phage antibodies derived from the mutation of each of fdCAT2scFvB18 and fdDOGKanscFvB18 in the ELISA. The ELISA conditions were such that the parent phage fdCAT2scFvB18 only generated weak signals. These phage antibodies giving strongly positive ELISA signals were enriched in further rounds by a factor of roughly 2.5 per round. Forty phage antibodies giving strongly positive signals were sequenced and they each displayed single mutations in six different positions in the scFvB18 nucleotide sequences, five of which reside in the light chain. More than 70% of the mutations occurred at positions 724 and 725 changing the first glycine in the J segment of the light chain (framework 4) to serine (in 21 cases) or aspartate (in 3 cases). The mutations found are shown in Table 9. The sequence of scFvB18 is shown in Figure 44(i) through 44(ii).- -

Please replace the paragraph bridging page 218 (starting at line 13) through page 219 (line 2) with the following rewritten paragraph:

- - The overall strategy for the PCR assembly is shown in fig.47 and is described in more detail below. For Fab assembly, the VH-CH1 and VK-CK or V lambda-C lambda light chains are amplified from first strand cDNA and gel purified. Heavy and light chain DNA are then combined together with linker DNA and flanking oligonucleotides in a new PCR reaction. This results in a full length Fab construct since the 5' end of the linker DNA is complementary to the 3' end of the CH1 domain and the 3' end of the linker is complementary to the 5' end of the light chain domain. The linker DNA contains terminal residues of the human CH1 domain, the bacterial leader sequence (pelB) for the light chain and the initial residues of the VK or V lambda light chain. Finally, after gel purification, the Fab construct is reamplified with flanking oligonucleotides containing restriction sites for cloning.- -

Please replace the paragraph bridging page 227 (starting at line 20) through page 228 (line 25) with the following rewritten paragraph:

- - The human hybridoma Fog-B has been previously described (N.C. Hughes-Jones et al Biochem, J. 268 135 (1990). It produces an IgG-1/lambda antibody which binds the Rh-D antigen. RNA was prepared from 10^7 hybridoma cells using a modified method of Cathala (as described in example 14) and 1st strand cDNA synthesized using specific immunoglobulin heavy and light chain primers (HuVH1FOR [example 40] and HuCλ FOR (5'-GGA ATT CTT ATG AAG ATT CTG TAG GGG CCA C-3')(SEQ ID NO:73)) as described in example 14. The VH gene was subsequently amplified from an aliquot of the 1st strand cDNA using HuVH4aBACK and HuVH1FOR. The Vλ gene was amplified using a Vλ primer specific for Fog-B (VλFog-B: 5'-AAC CAG CCA TGG CC AGT CTG TGT TGA CGC AGC C-3')(SEQ ID NO:74). The PCR conditions were as described in example 40. The PCR products were analyzed by running 5µl on a 2% agarose gel. The remainder was extracted twice with ether, twice with phenol/chloroform, ethanol precipitated and resuspended in 50µl of H_2O. The amplified VH DNA was digested with Pst1 and BstEII, and the amplified Vλ-Cλ DNA with Ncol and EcoR1. The fragments were purified on a 2% agarose gel, extracted using Geneclean, and sequentially ligated into the soluble expression vector pJM-1 Fab D1.3 (Fig 48(i)). Clones containing the correct insert were initially identified by restriction analysis and verified by assay of expressed soluble Fab (see example 23 for induction conditions). The Fog-B Fab cassette was amplified from pJM-1 by PCR using HuVH4BACK-Sfi and Hu Cλ-Not, digested with the appropriate restriction enzymes and ligated into pHEN1. Clones containing the correct insert were

identified initially by restriction analysis and subsequently by assay (see example 25 for induction conditions).- -

Please replace the paragraph bridging page 248 (starting at line 19) through page 249 (line 17) with the following rewritten paragraph:

- -The oligonucleotides mutL91,92, was prepared too randomise phenylalanine at position 91 (L91) and tryptophan at position 92 (L92) of the light chain. The oligonucleotides mutL32, was prepared to randomise tyrosine at light chain position 32 (L32) and the oligonucleotides mutH101 was prepared to randomise tyrosine at position 101 of the heavy chain (H101). mutL91,92:

5' CGT CCG AGG AGT ACT NNN NNN ATG TTG ACA GTA ATA 3' (SEQ ID NO:75)

mutL32:

5' CTG ATA CCA TGC TAA NNN ATT GTG ATT ATT CCC 3' (SEQ ID NO:76)

mutH101:

5' CCA GTA GTC AAG CCT NNN ATC TCT CTC TCT GGC 3'(SEQ ID NO:77)

(N represents a random insertion of equal amounts of A,C,G or T) in vitro mutagenesis of the phagemid vector, pCAT3scFvD1.3 (example 17) with the oligonucleotide mutL91,92 was carried out using an in vitro mutagenesis kit (Amersham). The resultant DNA was transformed by electroporation into TG1 cells using a Bio-Rad electroportor. 78,000 clones were obtained and these were scraped into 15mls of 2xTY/20% glycerol. This pool was called D1.3L91L92. Single stranded DNA was prepared by rescue with M13K07 as described in Sambrook et al, 1989 supra, and sequenced with the primer FDTSEQ1, using a Sequenase sequencing kit (United States Biochemical Corporation).- -

On page 252, please replace lines 22-29, with the following rewritten paragraph:

- - A dilution series was made on 10 clones which were analysed by ELISA in 6 of these clones the profile of binding to HEL was the same as the original clone (pCAT3SCFvD1.3) while the signal with TEL was increased (see figure 50(i) clone B1). In the remaining 4 clones, the increased signal with TEL was accompanied by a decrease in signal on HEL (see figure 50 clone A4).- -

Please replace the paragraph bridging page 258 (starting at line 22) through page 259 (line 5) with the following rewritten paragraph:

- - After 1 hour of incubation with mixing at room temperature, magnetic beads were recovered using a Dynal MPC-E magnetic desperation device. They were then washed in PBS containing 0.5% Tween 20, (3x10 minutes, 2x1 hour, 2x 10 minutes) and phage eluted by 5 minutes incubation in 50µl PBS containing 10mM dithiothreitol. The eluate was used to infect TG1 cells and the resulting colonies probed with the oligo NQ11CDR3
(5' AAACCAGGCCCCGTAATCATAGCC 3') (SEQ ID NO:80)
derived from CDR3 of the NQ11 antibody (This hybridises to pAbNO11 but not pAb D1.3).- -

Replace Table 10(i) on page 280 with the table on the following page:

Table 10(i) Oligonucleotide primers used for PCR of human immunoglobulin genes

Oligo Name	Sequence
Human VH Back Primers	
HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3' (SEQ ID NO:81)
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3' (SEQ ID NO:82)
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3' (SEQ ID NO:83)
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3' (SEQ ID NO:84)
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3' (SEQ ID NO:85)
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3' (SEQ ID NO:86)
HuVH1aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3' (SEQ ID NO:87)
HuVH2aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3' (SEQ ID NO:88)
HuVH3aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3' (SEQ ID NO:89)
HuVH4aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3' (SEQ ID NO:90)
HuVH5aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3' (SEQ ID NO:91)
HuVH6aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3' (SEQ ID NO:92)
Human JH Forward Primers	
HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3' (SEQ ID NO:93)
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3' (SEQ ID NO:94)
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3' (SEQ ID NO:95)
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3' (SEQ ID NO:96)
Human Heavy Chain Constant Region Primers	

Replace Table 10(ii) on page 281 with the table on the following page:

Table 10(ii)

HuIgG1-4CH1FOR	5'-GTC CAC CTT GGT GTT GCT GGG CTT-3' (SEQ ID NO:97)
HuIgMFOR	5'-TGG AAG AGG CAC GTT CTT TTC TTT-3' (SEQ ID NO:98)

Human Vκ Back Primers

HuVκ1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3' (SEQ ID NO:99)
HuVκ2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3' (SEQ ID NO:100)
HuVκ3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3' (SEQ ID NO:101)
HuVκ4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3' (SEQ ID NO:102)
HuVκ5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3' (SEQ ID NO:103)
HuVκ6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3' (SEQ ID NO:104)

Human Jκ Forward Primers

HuJκ1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3' (SEQ ID NO:105)
HuJκ2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3' (SEQ ID NO:106)
HuJκ3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'(SEQ ID NO:107)
HuJκ4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3' (SEQ ID NO:108)
HuJκ5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3' (SEQ ID NO:109)
HuJκ1BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3' (SEQ ID NO:110)
HuJκ2BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAG CTT GGT CCC-3' (SEQ ID NO:111)
HuJκ3BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3' (SEQ ID NO:112)
HuJκ4BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3' (SEQ ID NO:113)
HuJκ5BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3' (SEQ ID NO:114)

Human κ Constant Region Primers

Please replace Table 10(iii) on page 282 with the table on the next page:

Table 10(iii)

HuCκFOR	5'-AGA CTC TCC CCT GTT GAA GCT CTT-3' (SEQ ID NO:115)
HuCκFORNot1	5'-GAG TCA TTC TCG ACT TGC GGC CGC TTA TTA AGA CTC TCC CCT GTT GAA GCT CTT-3' (SEQ ID NO:116)
HuCκFORNot2	5'-GAG TCA TTC TCG ACT TGC GGC CGC AGA CTC TCC CCT GTT GAA GCT CTT-3' (SEQ ID NO:117)

Human λ Back Primers

HuVλ1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3' (SEQ ID NO:118)
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3' (SEQ ID NO:119)
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3' (SEQ ID NO:120)
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3' (SEQ ID NO:121)
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3' (SEQ ID NO:122)
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3' (SEQ ID NO:123)
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3' (SEQ ID NO:124)

Human λ Forward Primers

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3' (SEQ ID NO:125)
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3' (SEQ ID NO:126)
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3' (SEQ ID NO:127)

HuJλFORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3' (SEQ ID NO:128)
HuJλ2-3FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3' (SEQ ID NO:129)
HuJλ4-5FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACY TAA AAC GGT GAG CTG GGT CCC-3' (SEQ ID NO:130)

Human λ Constant Region Primers

Replace Table 10(iv) from page 283 with the table on the following page:

Table 10(iv)

HuCλFOR	5'-TGA AGA TTC TGT AGG GGC CAC TGT CTT-3' (SEQ ID NO:131)
HuCλFORNot1	5'-GAG TCA TTC TCG ACT TGC GGC CGC TTA TTA TGA AGA TTC TGT AGG GGC CAC TGT CTT-3'(SEQ ID NO:132
HuCλFORNot2	5'-GAG TCA TTC TCG ACT TGC GGC CGC TGC AGA TTC TGT AGG GGC TGT CTT-3' (SEQ ID NO:133)

Linker oligos

Reverse JH for scFv linker

RHuJH1-2	5'-GCA CCC TGG TCA CCG TCT CCT CAG GTG G-3' (SEQ ID NO:134)
RHuJH3	5'-GGA CAA TGG TCA CCG TCT CTT CAG GTG G-3' (SEQ ID NO:135)
RHuJH4-5	5'-GAA CCC TGG TCA CCG TCT CCT CAG GTG G-3' (SEQ ID NO:136)
RHuJH6	5'-GGA CCA CGG TCA CCG TCT CCT CAG GTG C-3' (SEQ ID NO:137)

Reverse IgG1-4CH1 primer for Fab linker

RhuIgG1-4CH1FOR	5'-AAG CCC AGC AAC ACC AAG GTG GAC-3' (SEQ ID NO:138)

Reverse Vκ for scFv linker

RhuVκ1aBACKFv	5'-GGA GAC TGG GTC ATC TGG ATG TCC GAT CCG CC-3' (SEQ ID NO:139)
RhuVκ2aBACKFv	5'-GGA GAC TGA GTC ATC ACA ACA TCC GAT CCG CC-3' (SEQ ID NO:140)
RhuVκ3aBACKFv	5'-GGA GAC TGC GTC AAC ACA ATT TCC GAT CCG CC-3' (SEQ ID NO:141)
RhuVκ4aBACKFv	5'-GGA GAC TGG GTC ATC ACG ATG TCC GAT CCG CC-3' (SEQ ID NO:142)
RhuVκ5aBACKFv	5'-GGA GAC TGC GTG AGT GTC GTT TCC GAT CCG CC-3' (SEQ ID NO:143)
RhuVκ6aBACKFv	5'-GGA GAC TGA GTC AGC ACA ATT TCC GAT CCG CC-3' (SEQ ID NO:144)

Reverse Vκ for Fab linker

Please replace Table 10(v) from pages 284 and 285 with the following table:

Table 10(v)

RHuVκ1aBACKFab	5'-GGA GAC TGG GTC ATC TGG ATG TCG GCC ATC GCT GG-3' (SEQ ID NO:145)
RHuVκ2aBACKFab	5'-GGA GAC TGC GTC ATC ACA ACA TCG GCC ATC GCT GG-3' (SEQ ID NO:146)
RHuVκ3aBACKFab	5'-GGA GAC TGC GTC AAC ACA ATT TCG GCC ATC GCT GG-3' (SEQ ID NO:147)
RHuVκ4aBACKFab	5'-GGA GAC TGG GTC ATC ACG ATG TCG GCC ATC GCT GG-3' (SEQ ID NO:148)
RHuVκ5aBACKFab	5'-GGA GAC TGC GTG AGT GTC GTT TCG GCC ATC GCT GG-3' (SEQ ID NO:149)
RHuVκ6aBACKFab	5'-GGA GAC TGC GTC AGC ACA ATT TCG GCC ATC GCT GG-3' (SEQ ID NO:150)

Reverse Vλ for svFv linker

RHuVλBACK1Fv	5'-GGC GGC TGC GTC AAC ACA GAC TGC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:151)
RHuVλBACK2Fv	5'-GCA GGC TGA GTC AGA GCA GAC TGC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:152)
RHuVλBACK3aFv	5'-GGT GGC TGA GTC AGC ACA TAG GAC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:153)
RHuVλBACK3bFv	5'-GGG TCC TGA GTC AGC TCA GAA GAC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:154)
RHuVλBACK4Fv	5'-GGC GGT TGA GTC AGT ATA ACG TGC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:155)
RHuVλBACK5Fv	5'-GAC GGC TGA GTC AGC ACA GAC TGC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:156)
RHuVλBACK6Fv	5'-TGG GGC TGA GTC AGC ATA AAA TTC GAT CCG CCA CCG CCA GAG-3' (SEQ ID NO:157)

Reverse Vλ for Fab linker

RHuVλBACK1Fab	5'-GGC GGC TGC GTC AAC ACA GAC TGG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:158)
RHuVλBACK2Fab	5'-GCA GGC TGA GTC AGA GCA GAC TGG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:159)
RHuVλBACK3aFab	5'-GGT GGC TGA GTC AGC ACA TAG GAG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:160)
RHuVλBACK3bFab	5'-GGG TCC TGA GTC AGC TCA GAA GAG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:161)
RHuVλBACK4Fab	5'-GGC GGT TGA GTC AGT ATA ACG TGG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:162)
RHuVλBACK5Fab	5'-GAC GGC TGA GTC AGC ACA GAC TGG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:163)
RHuVλBACK6Fab	5'-TGG GGC TGA GTC AGC ATA AAA TTG GCC ATC GCT GGT TGG GCA-3' (SEQ ID NO:164)

Please replace Table 11 on page 286 with the table on the next page:

Table 11. Deduced protein sequences of heavy and light chains selected from unimmunized library

Oxazolone binder

HEAVY CHAIN

VH15.4	QVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGIS	WVRQAPGQGLEWMG	WISAYNGNTKYAQKLQG	RVTMITDTSTSTAYMELRSLRSDDTAVYYCVR	LLPKRTATLH YYIDVWGKGT	(SEQ ID NO:165)

LIGHT CHAIN

VL15.4	NNYVS	WYQHLPGTAPNLLIY	DNNKRPS	GIPDRFSGSKSGTSATLGITGLQTGDEADYYC	GIWDGR	(SEQ ID NO:166)

BSA Binders

HEAVY CHAINS

VH3.5	QVQLVQSGGGVVQPGRSLRLSCAASGFTFS SYGMH	WVRQAPGKGLEWVA	VISYDGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	TGYSSGWGY FDYWGQGT	(SEQ ID NO:167)

LIGHT CHAINS

VL3.5	SSELTQDPAVSVALGQTVRITC QGDSLRSYYAS	WYQQKPGQAPVLVIY	GKNNRPS	GIPDRFSGSSSGNTASLTITGAQAEDEADYYC	NSRDSSGNH VVFGG	(SEQ ID NO:168)

Lysozyme binders:

HEAVY CHAINS

VH10.1	SLTCSVSGDSIS SGGYS	WIRQPSGKGLEWIG	SVHHSGPTYYNPSLKS	RVTMSVDTSKNQFSLKLKSVTAADTAMYFCAR	EGGSTWRSLYKH YYMDVWGK	(SEQ ID NO:169)
VH14.1	QVQLQESGPGLVKPSETLSLVCTVSGGSLS FSYWG	WIRQPPGKGLEWIG	YISHRGTDYNSSLQS	RVTISADTSKNQFSLKLSSVTAADTAVYYCAR	SFSNSFFFGY WGQGT	(SEQ ID NO:170)
VH13.1	QVQLVQSGAEVKKPGQSLMISCQGSGYSFS NYWIG	WVRQMPGKGLEWMG	IIYPGDSDTRYSPSFQG	QVTISADKSISTAYLHWSSLKASDTALYYCAR	LVGGTPAY WGQGT	(SEQ ID NO:171)
VH16.1	QVQLVQSGAEVKKPGQSLRISCKGAGYSFS TYWIG	WVRQMPGKGLEWMG	IIYPDDSDTRYSPSFEG	QVTISVDKSITTAYLHWSSLKA		(SEQ ID NO:172)

LIGHT CHAINS

VK10.1	EIVLTQSPSSLSASVGDRVTITC RASQSISNYLN	WYQQKPGKAPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTINSLQPEDFATYYC	QQTIISFP LTFGGG	(SEQ ID NO:173)
VL14.1	SSELTQDPAVSVAFGQTVRITC QGDSLRSSYAS	WYQQKPGQAPLLVIY	GENSRPS	GIPDRFSGSSSGNTASLTITGAQAEDEADYYC	NSRDSRGTHL EVFGG	(SEQ ID NO:174)
VL13.1	HVILTQPASVSGSPGQSITISC TGSSRDVGGYNYVS	WYQHHPGKAPKLLIS	EVTNRPS	GVSNRFSGSKSGNTASLTISGLQAEDEADYFC	ASYTSSKT YVFGG	(SEQ ID NO:175)
VL16.1	QSALTQPASVSGSPGQSITISC SGSSSDIGRYDYVS	WYQHYPDKAPKLLIY	EVKHRPS	GISHRFSASKSGNTASLTISELQPGDEADYYC	ASYT	(SEQ ID NO:176)

Applicants believe the application is now in a condition for allowance. Favorable reconsideration of the application is respectfully requested. The Examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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Appendix to the Specification

On page 1, line 3, after the title, insert the following paragraph:

- - This is a continuation of U.S. Application Serial No. 08/484,893, filed June 7, 1995 (allowed), which in turn is a continuation of U.S. Application Serial No. 07/971,857, filed January 8, 1993 (now U.S. Patent No. 5,969,108), which in turn is the U.S. national phase of PCT/GB91/01134, filed 10 July 1991.- -

On page 35, between lines 12 and 13, insert the following subtitle

- - Summary of the Invention - -

Replace the paragraph bridging pages 42 and 43 with the following rewritten paragraph:

- - The rgdp may be a bacteriophage, the host a bacterium, and said component of the rgdp a capsid protein for the **[bacterophage]** **bacteriophage**. The phage may be a filamentous phage. The phage may be selected from the class I phages fd, M13, f1, If1, lke, ZJ/Z, Ff and the class II phages Xf, Pf1 and Pf3. The phage may be fd or a derivative of fd. The derivative may be tetracycline resistant. The said sbp member or polypeptide chain thereof may be expressed as a fusion with the gene III capsid protein of phage fd or its counterpart in another filamentous phage. The sbp member or polypeptide chain thereof may be inserted in the N-terminal region of the mature capsid protein downstream of a secretory leader peptide. The sequence may be inserted after amino acid +1 of the mature protein. The site for insertion may be flanked by short sequences corresponding to sequences which occur at each end of the nucleic acid to be inserted. For example where 4 the protein domain is an immunoglobulin domain, the insertion site in the phage may be flanked by nucleotide sequences which code for the first five amino acids and the last five amino acids of the Ig domain. Such flanking nucleotide sequences are shown in figure 4(2) B and C, wherein the site-flanking nucleotide sequences encode amino acid sequences QVQLQ **(SEQ ID NO:1)** and VTVSS **(SEQ ID NO:2)** which occur at either end of the VH domain, or QVQLQ **(SEQ ID NO:1)** and LEIKR **(SEQ ID NO:3)** which occur at either end of the Fv (combined VH + VL) domain. Each of these sequences flanking the insertion site may include a suitable cleavage site, as shown in **[Fig 4] Figure 4(i) and Figure 4(ii)**. - -

Please replace the paragraph starting at page 58, line 4, with the following rewritten paragraph:

--The applicants have also devised a series of novel selection techniques that are practicable only because of the unique properties of rgdps. The general outline of some screening procedures is illustrated in figure 2**(i) and figure 2(ii)** using pAbs as an example type of rgdp.--

Please replace the paragraph beginning at page 58, line 10, with the following rewritten paragraph:

--The population/library of pAbs to be screened could be generated from immunised or other animals; or be created in vitro by mutagenising pre-existing phage antibodies (using techniques well-known in the art such as oligonucleotide directed mutagenesis (Sambrook, J., *et al.*, 1989 Molecular Cloning a Laboratory Manual, Cold Spring Harbor Laboratory Press). This population can be screened in one or more of the formats described below with reference to figure 2**(i) and figure 2(ii)**, to derive those individual pAbs whose antigen binding properties are different from sample c.--

On page 65, immediately following line 20, please insert the following title:

- - Brief Description of the Drawings- -

On page 65, lines 23-29, replace the paragraph with the following rewritten paragraph:

- - **[Figure 2 shows]** **Figures 2(i) and 2(ii) show** schematically selection techniques which utilise the unique properties of pAbs; 2(i) shows a binding/elution system; and (2ii) shows a competition system (p=pAb; ag=antigen to which binding by pAb is required; c=competitor population e.g. antibody, pAb, ligand; s=substrate (e.g. plastic beads etc); d=detection system.- -

On page 66, lines 5-23, replace the paragraph with the following rewritten paragraph:

- - **[Figure 4 shows]** **Figures 4(i)-4(ii) show** the nucleotide sequences for the oligonucleotides and vectors. All sequences are drawn 5' to 3' and are numbered according to

Beck et al., 1978, Nucl. Acid Res., 5: 4495-4503. **[4.1]** **4(i)** shows the sequences of the oligonucleotides used for mutagenesis (oligo's 1 and 2) or sequencing (oligo 3). The sequences shown were synthesized on an Applied Biosystems, oligonucleotide synthesizer and are complementary to the single stranded form of fd-tet (they are in the anti-sense form with respect to gene III). **[4.2]** **4(ii)** shows the sequences of the various constructs around the gene III insertion site. These sequences are drawn in the sense orientation with respect to gene III; (A) fd-tet (and fdTδBst) (B) fdTPs/Bs and (C) fdTPs/Xh. The key restriction enzyme sites are shown along with the immunoglobulin amino acids contributed by the vectors, (amino acid single letter code is used, see Harlow, E., and Lane, D., 1988 supra.).- -

On page 68, lines 8-14, please replace the paragraph with the following rewritten paragraph:

- - **[Figure 12 shows]** **Figures 12a-12b show** oligonucleotide probing of affinity purified phage. 10^{12} phage in the ratio of 1 pAb (D1.3) in 4 x 10^4 fdTPS/Bs phages were affinity purified and probed with an oligonucleotide specific for pAb (D1.3) A is a filter after one round of affinity purification (900 colonies total) and B is a filter after two rounds (372 colonies total).- -

On page 69, lines 1-5, replace the paragraph with the following rewritten paragraph:

- -Figure **[16(1)]** **16(i)** shows the structure of gene III and the native BamHI site into which a scFv coding sequence was inserted in example 13 and figure **[16(2)]** **16(ii)** shows the natural peptide linker sites A and B for possible insertion of scFv coding sequences.- -

On page 70, lines 11-14, replace the paragraph with the following rewritten paragraph:

- - **[Figure 23 shows]** **Figures 23(i)-Fig. 23(ii) show** the digestion pattern seen when individual clones, selected at random from a library of single chain Fv antibody genes derived from an immunised mouse; are digested with BstN1.- -

On page 70, lines 23-25, please replace the paragraph with the following rewritten paragraph:

- - **[Figure 26]** <u>Fig. 26A</u> shows **[a)]** the phagemid pHEN1 a derivative of pUC119 described in example 24; and **[b)]** the cloning sites in the phagemid pHEN.- -

On page 72, lines 16-24, please replace the paragraph with the following rewritten paragraph:

- - **[Figure 35 is a Western Blot]** <u>**Figures 35A-35B are Western Blots**</u> showing ultrafiltration of phage-enzyme 100µl of 50 fold concentrate of phage (representing 5mls of culture supernatant) was centrifuged through ultrafiltration membranes with nominal molecular weight retention of 300,000 daltons. Western blots of flow through and retentate fractions were detected with anti-alkaline phosphatase antiserum. The equivalent of 800µl of original culture supernatant was run on the gel.- -

On page 72, lines 25-28, please replace the paragraph with the following rewritten paragraph:

- -**[A]** <u>**Fig. 35A.**</u> Phage were grown in TG1 cells. a) fd-phoAla166 before ultrafiltration (short exposure). b) fd-phoAla166 before ultrafiltration. c) fd-phoAla166 material retained on ultrafiltration membrane.- -

On page 73, lines 1-6, please replace the paragraph with the following rewritten paragraph:

- - **[B]** <u>**Fig. 35B**</u>. Phage were grown in KS272 cells. a) fd-phoAla166 before ultrafiltration. b) fd-phoAla166 material retained on ultrafiltration membrane. c) fdCAT2. d) fdCAT2 mixed with purified alkaline phosphatase before ultrafiltration. e) Retentate from sample d. f) Flow through from sample d.- -

On page 74, lines 19-21, please replace the paragraph with the following rewritten paragraph:

- -**[Figure 38]** **Figures 38A-38B.** Western blot of PEG precipitated phage used in ELISA probed with anti-g3p. Free g3p and the g3p-scFvD1.3 fusion bands are arrowed. - -

On page 75, lines 8-15, please replace the paragraph with the following rewritten paragraph:

- - **[Panel A]** **Fig. 38A** samples contain the equivalent of 8μl of phagemid culture supernatant per track, and 80μl of the fd supernatant (10-fold lower phage yield than the phagemid). **[Panel B]** **Fig. 38B** phagemid samples are those used in **[panel A] Fig. 38A** at a five-fold higher sample loading (equivalent to 40μl of culture supernatant per track) to enable visualisation of the fusion band in samples rescued with parental M13K07.- -

On page 76, lines 8-9, please replace the paragraph with the following rewritten paragraph:

- -**[Figure 44 shows]** **Figures 44(i)-(ii) show** the DNA sequence of scFv B18 (anti-NP).- -

On page 76, lines 23-29, please replace the paragraph with the following rewritten paragraph:

- - **[Figure 48. Shows A.]** **Figure 48(i) shows** a map of plasmid pJM1-FabD1.3 which is used for the expression of soluble human Fab fragments and as a template for the synthesis of linker DNA for Fab assembly. **[B.]** **Figure 48(ii) is** a schematic representation of sequences encoding a Fab construct. **[C]** **Fig. 48(iii)** shows the sequence of DNA template for the synthesis of linker DNA for Fab assembly.- -

On page 77, lines 5-13, please replace the paragraph with the following rewritten paragraph:

- - **[Figure 50]** **Figures 50(i)-(ii).** ELISA assay of phage antibodies using plates coated with turkey egg lysogyme. Two clones B1 and A4 are shown derived by mutagenesis and

selection from pAbD1.3 (example 45). Concentration (x axis) refers to the concentration of phage for each sample relative to the concentration in culture supernatant. B1 has raised binding to turkey egg lysogyme compared to D1.3. A4 has reduced binding to hen egg lysogyme compared to D1.3.- -

On page 94, lines 12-23, please replace the paragraph with the following rewritten paragraph:

- - In vitro mutagenesis of fdTδBst was used to generate vectors having appropriate restriction sites that facilitate cloning of antibody fragments downstream of the gene III signal peptide and in frame with the gene III coding sequence. The oligonucleotide directed mutagenesis system version 2 (Amersham International) was used with oligo 1 (figure 4(i)) to create fdTPs/Bs (to facilitate cloning of VH fragments). The sequence offdTPs/Bs (figure 4(i)) was confirmed using the sequenase version 2.0 kit (USB Corp., PO Box 22400, Cleveland, Ohio, 44122, UsA.) with oligo 3 (figure 4) as a primer.- -

On page 102, lines 1-12, please replace the paragraph with the following rewritten paragraph:

- - The oligonucleotide:
5'ACT TTC AAC AGT TTC TGC GGC CGC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG TGC ACT GTG AGA ATA GAA 3' **(SEQ ID NO:4)** was synthesised (supra fig 4 legend) and used to mutagenise fdTPs/Xh using an in vitro mutagenesis kit from Amersham International as described in example 1, to create fd-CAT2. The sequence of fd-CAT2 was checked around the site of manipulation by DNA sequencing. The final sequence around the insertion point within gene III is shown in figure 8. N.B. fdCAT2 is also referred to herein by the alternative terminologies fd-tet-DOG1 and fdDOG1.- -

In the paragraph bridging pages 105-106, please replace the paragraph with the following rewritten paragraph:

- - The starting point for this example was the clone Fab D1.3 in pUC19, a map of which is shown in figure 10. The regions hybridising with the oligonucleotides KSJ6 and 7 below are shown underlined in fig 10. The sequence encoding the VH-CH1 region (defined at the 5' and 3' edges by the oligonucleotides KSJ6 and 7 below) was PCR amplified from Fab D1.3 in pUC19 using oligonucleotides KSJ 6 and 7, which retain the Pst I site at the 5' end and introduce a Xho I site at the 3' end, to facilitate cloning into fd CAT2. The sequences for the oligonucleotides KSJ6 and 7 are shown below. The underlined region of KSJ7 shows the portion hybridising with the sequence for D1.3. KSJ6:5' AGG TGC AGC TGC AGG AGT CAG G 3' **(SEQ ID NO:5)** KSJ7: 5' GGT GAC CTC GAG TGA AGA TTT GGG CTC AAC TTT C 3' **(SEQ ID NO:6)**- -

Please replace the paragraph bridging pages 108 and 109, with the following rewritten paragraph:

- -The applicant purified pAb (D1.3) (originally called fdTscFvD1.3 in example 2) from mixtures using antigen affinity columns. pAb (D1.3) was mixed with vector fd phage (see table 1) and approximately 10^{12} phage passed over a column of lysozyme-Sepharose (prepared from cyanogen bromide activated sepharose 4B (Pharmacia, Milton Keynes, Bucks, UK.) according to the manufacturers instructions. TG1 cells were infected with appropriate dilutions of the elutes and the colonies derived, were analysed by probing with an oligonucleotide that detects only the pAb (D1.3) see Table 1 and **[Fig. 12]** **Figs. 12A and 12B**. A thousand fold enrichment of pAb(D1.3) was seen with a single column pass. By growing the enriched phage and passing it down the column again, enrichments of up to a million fold were seen. - -

On page 109, lines 15-26, please replace the paragraph with the following rewritten paragraph:

- - Enrichment was also demonstrated using purely immunological criteria. For example, 10^{12} phage (at a ratio of 1 pAb (D1.3) to 4 x 10^6 fdTPs/Bs) was subjected to two rounds of affinity selection, and then 26 colonies picked and grown overnight. The phage was then assayed for lysozyme binding by ELISA (as example 6). Five colonies yielded phage with lysozyme binding activities, see table 1, and these were shown to encode the scFv (D1.3) by PCR screening

(example 13, using 30 cycles of 1 minute at 92°C, 1 minute at 60°C, 1 minute at 72°C using CDR3PCR1 and oligo 3 **[(fig. 4)]** **(Fig. 4(i))** as primers).- -

On page 110, lines 4-24, please replace the paragraph with the following rewritten paragraph:

- - Approximately 10^{12} phage particles in 1ml MPBS were loaded onto a 1 ml lysozyme-Sepharose affinity column which had been prewashed in MPBS. The column was washed in turn with 10 ml PBS; then 10 ml 50 mM Tris-HCl, 500 mM Nacl pH 7.5; then 10ml 50 mM Tris-HCl 500 mM NaCl pH 8.5; then 5 mls 50 mM Tris-HCl, 500 mM NaCl pH 9.5 (adjusted with triethylamine) and then eluted with 5 ml 100 mM triethylamine. The eluate was neutralised with 0.5 M sodium phosphate buffer pH 6.8 and the phage plated for analysis. For a second round of affinity chromatography, the first column eluate was plated to about 30,000 colonies per petri dish. After overnight growth, colonies were then scraped into 5 ml 2 x TY medium, and a 20 µl aliquot diluted into 10 ml fresh medium and grown overnight. The phage was PEG precipitated as described above, resuspended in 1 ml MPBS and loaded onto the column, washed and eluted as above.

Oligonucleotides sythesised: CDR3PCR1 5'TGA GGA C(A or T) C(A or T) GC CGT CTA CTA CTG TGC 3' **(SEQ ID NO:7)**- -

On page 113, lines 8-22, please replace the paragraph with following rewritten paragraph:

- - 3 x 10^{10} phage in 10 mls of PBSM at the ratios of pAb D1.3 to pAb NQ11 shown in table 2 were passed over a 1 ml lysozyme Sepharose column. Washing, elution and other methods were as described in example 8 unless otherwise stated. Eluates from the columns were used to infect TG1 cells which were then plated out. Colonies were probed with a probe which distinguishes pAb D1.3 from pAb NQ11. The sequence of this oligonucleotide (D1.3CDR3A) is:-

5'GTA GTC AAG CCT ATA ATC TCT CTC 3' **(SEQ ID NO:8)** Table 2 presents the data from this experiment. An enrichment of almost 1000 fold was achieved in one round and an

enrichment of over a million fold in two rounds of purification. This parallels the result described in example 8.- -

Replace the paragraph bridging pages 113-114, with the following rewritten paragraph:

- - As an example of the expression of a functional enzyme on the bacteriophage surface, the applicants have chosen bacterial alkaline phosphatase, an enzyme that normally functions as a dimer (McCracken, S. and Meighen, E., J. Biol. Chem. 255, p2396-2404, (1980)). The oligonucleotides were designed to generate a PCR product with an Apa L1 site at the 5' end of phoA gene and a Not 1 site at its 3' end, thus facilitating cloning into fd-CAT 2 to create a gene III fusion protein. The oligonucleotides synthesised were: phoA1:5' TAT TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG 3' **(SEQ ID NO:9)** and, phoA2:5' ACA TGT ACA TGC GGC CGC TTT CAG CCC CAG AGC GGC TTT **[C3']** **C 3' (SEQ ID NO:10).** The sequence of the phoA gene is presented in Chang C. N. et al., Gene 44, p121-125 (1986). The plasmid amplified (pEK86) contains an alkaline phosphate gene which differs from the sequence of Chang et al, by a mutation which converts arginine to alamine at position 166.- -

Please replace the paragraph on page 118, lines 7-21, with the following rewritten paragraph:

- - DNA fragments encoding scFv's from D1.3 or NQ11 were generated by PCR using the primers shown below. These primers were designed to generate a fragment with BamH1 sites near both the terminii, to enable cloning into the BamH1 site of gene3 (see figure **[16(1)]** **16(i)**). The oligonucleotides used, also ensure that the resulting PCR product lacks Pst1 and Xhol restriction sites normally used for manipulating the scFv's (see figure 16(1)). This will facilitate subsequent manipulation of a second antibody fragment in the usual way at the N terminus of gene 3. The oligonucleotides used were:-

G3Baml 5'TTT AAT GAG GAT CCA CAG GTG CAG CTG CAA GAG 3' **(SEQ ID NO:11)**

G3Bam2 5'AAC GAA TGG ATC CCG TTT GAT CTC AAG CTT 3' **(SEQ ID NO:12)**.- -

Please replace the paragraph on page 120, lines 1-14, with the following rewritten paragraph:

- - It may be possible to clone into alternative sites to retain binding activity. The peptide repeats present in gene III may provide such a site (figure 16 blocks A and B). This can be done by inserting a BamHl site and using the PCR product described above. To facilitate this, the natural BamHl site was removed by mutagenesis with the oligonucleotide G3mutδBam shown below (using an in vitro mutagenesis kit (Amersham International)):-

G3mutδBam 5' CA AAC GAA TGG GTC CTC CTC ATT A 3' **(SEQ ID NO:13)**

The underlined residue replaces an A residue, thereby removing the BamHl site. DNA was prepared from a number of clones and several mutants lacking BamHl sites identified by restriction digestion.- -

Please replace the paragraph on page 120, lines 15-22, with the following rewritten paragraph:

- -The oligonucleotide G3 Bamlink was designed to introduce a BamHl site at a number of possible sites within the peptide linker sites A and B, see figure **[16(2)]16(ii)**. The sequence of the linker is: Bamlink 5'CC (G or A) CC ACC CTC GGA TCC (G or A) CC ACC CTC 3' **(SEQ ID NO:14).** Its relationship to the peptide repeats in gene III is shown in figure 16.- -

Replace the paragraph on page 120, lines 1-16 with the following paragraph:

- - 1. cDNA is prepared from spleen RNA from an appropriate mouse and the VH and VLK repertories individually amplified. Separately, primers reverse and complementary to VH1FOR-2 (domain 1) and VLK2BACK (domain 2) are used to amplify an existing scFv-containing DNA by PCR. (The term FOR refers to e.g. a primer for amplification of sequences on the sense strand resulting in antisense coding sequences. The term BACK refers to e.g. a primer for amplification of sequences on the antisense strand resulting in sense coding sequences). This generates a 'linker' molecule encoding the linker with the amino

acid sequence (1 letter code) $(GGGGS)_3$ **(SEQ ID NO:15)** which overlaps the two primary (VH and VLK) PCR products. - -

Please replace the paragraph on page 126, lines 14-21, with the following rewritten paragraph:

- - The primers anneal to the 3' end. Examples of kappa light chain primers are MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (provided under 'Primer Sequences' below) and examples of heavy chain primers are MIGG1, 2 (CTG GAC AGG GAT CCA GAG TTC CA) **(SEQ ID NO:16)** and MIGG3 (CTG GAC AGG GCT CCA TAG TTC CA) **(SEQ ID NO:17)** which anneal to CH1.- -

Please replace the paragraph beginning at page 127, line 28, with the following rewritten paragraph:

--Purify on a 2% 1mp (low melting point agarose/TAE (tris-acetate EDTA)gel and extract the DNA to 20 µl H_2O per original PCR using a **[Geneclean]** **GENECLEAN** kit (see earlier; Bio101, La Jolla CA, USA) in accordance with the manufacturers instructions.--

Please replace the material bridging page 132 (starting at line 10) through page 133 (line 8) with the following:

- - Primer sequences

Primary PCR oligos (restrictions sites underlined):

VH1FOR-2 TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC **(SEQ ID NO:18)**

VH1BACK AGG TSM ARC TGC AGS AGT CWG G **(SEQ ID NO:19)**

MJK1FONX CCG TTT GAT TTC CAG CTT GGT GCC **(SEQ ID NO:20)**

MJK2FONX CCG TTT TAT TTC CAG CTT GGT CCC **(SEQ ID NO:21)**

MJK4FONX CCG TTT TAT TTC CAA CTT TGT CCC **(SEQ ID NO: 22)**

MJK5FONX CCG TTT CAG CTC CAG CTT GGT CCC **(SEQ ID NO:23)**

VK2BACK GAC ATT GAG CTC ACC CAG TCT CCA **(SEQ ID NO:24)**

Ambiguity codes M = A or C, R = A or G, S = G or C,

W = A or T

PCR oligos to make linker:

LINKFOR TGG AGA CTC GGT GAG CTC AAT GTC **(SEQ ID NO:25)**

LINKBACK GGG ACC ACG GTC ACC GTC TCC TCA **(SEQ ID NO:26)**

For adding restriction sites:

HBKAPA10 CAT GAC CAC AGT GCA CAG GTS MAR CTG CAG SAG TCW GG **(SEQ ID NO:27)**

JKINOT10 GAG TCA TTC TGC GGC CGC CCG TTT GAT TTC CAG CTT GGT GCC **(SEQ ID NO:28)**

JK2NOT10 GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAG CTT GGT CCC **(SEQ ID NO:29)**

JK4NOT10 GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAA CTT TGT CCC **(SEQ ID NO:30)**

JK5NOT10 GAG TCA TTC TGC GGC CGC CCG TTT CAG CTC CAG CTT GGT CCC **(SEQ ID NO:31)**- -

On page 134, please replace lines 7-11, with the following rewritten paragraph:

- - RPDGF3 5' CAC AGT GCA CTG GTC GTC ACA CCC CCG GGG CCA GAG CTT GTC CTC AAT GTC TCC AGC ACC TTC GTT CTG 3' **(SEQ ID NO:32)**

RPDGF2 5' GAT CTC GAG CTT AAA GGG CAA GGA GTG TGG CAC 3' **(SEQ ID NO:33)**- -

On page 139, please replace lines 1-7, with the following rewritten paragraph:

- -Gene III from fd-CAT2 (example 5) and the gene III scFv fusion from fd-CAT2 scFvD1.3 was PCR-amplified using the primers A and B shown below:
Primer A: TGC GAA GCT TTG GAG CCT TTT TTT TTG GAG ATT TTC AAC G **(SEQ ID NO:34)**
Primer B: CAG TGA ATT CCT ATT AAG ACT CCT TAT TAC GCA GTA TGT TAG C **(SEQ ID NO:35)**- -

On Page 145, please replace lines 1-26 with the following rewritten paragraph:

- - Vector fdCAT2 was extensively digested with Not1 and ApaL1., purified by electroelution (Sambrook et al.a989 supra) and 1 μg ligated to 0.5 μg (5 μg for the hierarchial libraries: see example 22) of the assembled scFv genes in 1 ml with 8000 units T4 DNA ligase (New England Biolabs). The ligation was carried out overnight at 16°C. Purified ligation mix was electroporated in six aliquots into MC1061 cells (W. J. Dower, J. F. Miller & C. W. Ragsdale Nucleic Acids Res. 16 6127-6145 1988) and plated on NZY medium (Sambrook et al. 1989 supra) with 15μg/ml tetracycline, in 243x243 mm dishes (Nunc): 90-95% of clones contained scFv genes by PCR screening. Recombinant colonies were screened by PCR (conditions as in example 7 using primers VH1BACK and MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (see example 14) followed by digestion with the frequent cutting enzyme BstN1 (New England Biolabs, used according to the manufacturers instructions). The library of $2x10^5$ clones appeared diverse as judged by the variety of digestion patterns seen in Figure 23**(i) and Figure 23(ii)**, and sequencing revealed the presence of most VH groups (R. Dildrop, Immunol. Today 5 85-86. 1984) and VK subgroups (Kabat. E.A. et al. 1987 supra) (data not shown). None of the 568 clones tested bound to phOx as detected by ELISA as in example 9.- -

Please replace the material bridging page 147 (starting at line 27) through page 148 (line 11) with the following rewritten paragraph:

- - To sequence clones, template DNA was prepared from the supernatants of 10 ml cultures grown for 24 hours, and sequenced using the dideoxy method and a Sequenase kit

(USB), with primer LINKFOR (see example 14) for the VH genes and primer fdSEQ1 (5'-GAA TTT TCT GTA TGA GG **-3') (SEQ ID NO:36)** for the Vk genes. Twenty-three of these hapten-binding clones were sequenced and eight different VH genes (A to H) were found in a variety of pairings with seven different Vk genes (a to g) (Fig. 24). Most of the domains, such as VH-B and Vk-d were 'promiscuous', able to bind hapten with any of several partners.- -

Please replace the material bridging page 148 (starting at line 10) through page 149 (line 9) with the following rewritten paragraph:

- -The sequences of the V-genes were related to those seen in the secondary response to phOx, but with differences (Fig. 24). Thus phOx hybridomas from the secondary response employ somatically mutated derivatives of three types of Vk genes - Vkoxl. 'Vkox-like' and Vk45.1 genes (C. Berek, G. M. Griffiths & C. Milstein Nature 316 412-418 (1985). These can pair with VH genes from several groups, from Vkoxl more commonly pairs with the VHoxl gene (VH group 2. R.Dildrop uupra). Vkoxl genes are always, and Vkox-like genes often, found in association with heavy chains (including VHoxl) and contain a short five residue CDR3, with the sequence motif Asp-X-Gly-X-X **(SEQ ID NO:37)** in which the central glycine is needed to create a cavity for phOx. In the random combinatorial library however, nearly all of the VH genes belonged to group 1, and most of the Vk genes were ox-like and associated with VH domains with a five residue CDR3, motif Asp/Asn-X-Gly-X-X **(SEQ ID NO:38)** (Fig. 24). Vkoxl and VHoxl were found only once (Vk-f and VH-E), and not in combination with each other. Indeed Vk-f lacks the Trp91 involved in phOx binding and was paired with a VH (VH-C) with a six residue CDR3.- -

Please replace the material bridging page 151 (starting at line 9) through page 152 (line 8) with the following rewritten paragraph:

- -The promiscuity of the VH-B and Vk-d domains prompted the applicants to force further pairings, by assembling these genes with the entire repertoires if either Vk or VH genes from the same immunised mice. The resulting 'hierarchical' libraries, (VH-B x Vk-rep and VH-rep x Vk-d), each with $4x10^7$ members, were subjected to a round of selection and hapten-

binding clones isolated (Table 4). As shown by ELISA, most were strong binders. By sequencing twenty-four clones from each library, the applicants identified fourteen new partners for VH-B and thirteen for Vk-d (Fig. 24). Apart from VH-B and Vk-c, none of the previous partners (or indeed other clones) from the random combinatorial library was isolated again. Again the Vk genes were mainly ox-like and the VH genes mainly group 1 (as defined in Dildrop, R. 1984 supra), but the only examples of Vkoxl (Vk-h, -p, -q and -r) have Trp91, and the VH-CDR3 motif Asp-X-Gly-X-X **(SEQ ID NO:37)** now predominates. Thus some features of the phOx hybridomas seemed to emerge more strongly in the hierarchial library. The new partners differed from each other mainly by small alterations in the CDRs, indicating that much of the subtle diversity had remained untapped by the random combinatorial approach. More generally it has been shown that a spectrum of related antibodies can be made by keeping one of the partners fixed and varying the other, and this could prove invaluable for fine tuning of antibody affinity and specificity.- -

Please replace the material bridging page 153 (starting at line 8) through page 154 (line 26) with the following rewritten paragraph:

- - Clones VH-B/Vk-b and VH-B/Vk-d were reamplified with MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (see example 14) and VH1BACK-SfiI (5'-TCG CGG CCC AGC CGG CCA TGG CC(G/C) AGG T(C/G)(A/C) A(A/G)C TGC AG(C/G) AGT C(A/T)G G-3') **(SEQ ID NO:39)**, a primer that introduces an SfiI site (underlined) at the 5' end of the VH gene. VH-B/Vk-d was cloned into a phagemid e.g. pJM1 (a gift from A. Griffiths and J. Marks) as an SfiI-NotI cassette, downstream of the pelB leader for periplasmic secretion (M. Better at al. supra), with a C-terminal peptide tag for detection (see example 24 and figure), and under th control of a P_L promoter (H. Shimatake & M. Rosenberg Nature 292 128-132 1981). The phagemid should have the following features: a) unique SfiI and Not1 restriction sites downstream of a pelB leader; b) a sequence encoding a C-terminal peptide tag for detection; and c) a λ P_L promoter controlling expression. 10 litre cultures of E.coli N4830-1 (M. E. Gottesman, S. Adhya & A. Das J.Mol.Biol 140 57-75 1980) harbouring each phagemid were induced as in K. Nagai & H. C. Thogerson (Methods Enzymol 153 461-481 1987) and supernatants precipitated

with 50% ammonium sulphate. The resuspended precipitate was dialysed into PBS + 0.2 mM EDTA (PBSE), loaded onto a 1.5ml column of phOx:Sepharose and the column washed sequentially with 100 ml PBS: 100 ml 0.1 M Tris-HC1, 0.5 M NaC1, pH 8.0: 10ml 50 mM citrate, pH 5.0: 10 ml 50 mM citrate, pH4.0, and 20 ml 50 mM glycine, pH 3.0. scFv fragments were eluted with 50 mM glycine, pH 2.0, neutralised with Tris base and dialysed against PBSE. VH-B/Vk-b was cloned into a phagemid vector based on pUC119 encoding identical signal and tag sequences to pJM1, and expression induced at 30°C in a 10 litre culture of E.coli TG1 harbouring the phagemid as in D. de Bellis & I. Schwartz (1980 Nucleic Acids Res 18 1311). The low affinity of clone VH-B/Vk-b made its purification on phOx-Sepharose impossible. Therefore after concentration by ultrafiltration (Filtron, Flowgen), the supernatant (100 ml of 600 ml) was loaded onto a 1 ml column of protein A-Sepharose cpoupled (E. Harlow & D. Lane 1988 supra) to the monoclonal antibody 9E10 (Evan, G. I. et al. Mol.Cell Biol.5 3610-3616 1985) that recognises the peptide tag. The column was washed with 200 ml PBS and 50 ml PBS made 0.5 M in NaC1. scFv fragments were eluted with 100 ml 0.2M glycine, pH 3.0, with neutralisation and dialysis as before.- -

Please replace the material bridging page 154 (starting at line 27) through page 155 (line 28) with the following rewritten paragraph:

- - The Kd ($1.0 \pm 0.2 \times 10^{-8}$ M) for clone VH-B/Vk-d was determined by fluorescence quench titration with 4-E-amino-butyric acid methylene 2-phenyl-oxazol-5-one (phOx-GABA Co. Makela et al, 1978 supra). Excitation was at 280 nm, emission was monitored at 340 nm and the K_d calculated. The K_d of the low affinity clone VH-B/Vk-b was determined as $1.8 \pm 0.3 \times 10^{-5}$ M (not shown). To minimise light adsorption by the higher concentrations of phOx-GABA required, excitation was at 260 nm and emission was monitored at 304 nm. In addition the fluorescence values were divided by those from a parallel titration of the lysozyme binding Fv fragment D1.3. The value was calculated as in H. N. Eisen Meth.Med.Res. 10 115-121 1964. A mixture of clones VH-B/Vk-b and VH-B/Vk-d, 7×10^{10} TU phage in the ratio 20 VH-B/Vk-b : 1 VH-B/Vk-d were loaded onto a phOx-BSA-Sepharose column in 10 ml MPBS and eluted as above. Eluted phage were used to reinfect E.coli TG1, and phage produced and harvested as

before. Approximately 10^{11} TU phage were loaded onto a second affinity column and the process repeated to give a total of three column passes. Dilutions of eluted phage at each stage were plated in duplicate and probed separately with oligonucleotides specific for Vk-b (5'-GAG CGG GTA ACC ACT GTA CT-3')**(SEQ ID NO:40)** or Vk-d (5'-GAA TGG TAT AGT ACT ACC CT-3')**(SEQ ID NO:41)**. After these two rounds, essentially all the eluted phage were VH-B/Vk-d (table 4). Therefore phage antibodies can be selected on the basis of the antigen affinity of the antibody displayed.

Example 24 - -

On page 156, please replace lines 23 through 27 with the following rewritten paragraph:

- - G3FUFO,5'-CAG TGA ATT CTT ATT AAG ACT CCT TAT TAC GCA GTA TGT TAG-3' **(SEQ ID NO:42)**;

G3FUBA,5'-TGC GAA GCT TTG GAG CCT TTT TTT TTG GAG ATT TTC AAC G-3' **(SEQ ID NO:43)**;

Example 25 - -

On page 156, please replace lines 4-21 with the following rewritten paragraph:

- - The phagemid pHEN1 (figure 26**(a)**) is a derivative of pUC119 (Vieira, J. & Messing, J. Methods Enzymol 153 pp 3-11, 1987). The coding region of g3p from fdCAT2, including signal peptide and cloning sites, was amplified by PCR, using primers G3FUFO and G3FUBA (given below) (which contain EcoRI and HindIII sites respectively), and cloned as a HindIII-EcoRI fragment into pUC119. The HindIII-NotI fragment encoding the g3p signal sequence was the replaced by a pelB signal peptide (Better, M. et al. Science 240 1041-1043, 1988) with an internal SfiI site, allowing antibody genes to be cloned as fiI-NotI fragments. A peptide tag, c-myc, (Munro, S. & Pelham, H. Cell 46 291-300, 1986) was introduced directly after the NotI site by cloning an oligonucleotide cassette, and followed by an amber codon introduced by site-directed mutagenesis using an in vitro mutagenesis kit (Amersham International) (figure **[26b]** **26(b)**).- -

On page 158, please replace lines 12 through 28 with the following rewritten paragraph:

- - VH1BACKAPA, 5'-CAT GAC CAC AGT GCA CAG GT(C/G) (A/C)A(A/G) CTG CAG (C/G)AG TC(A/T) GG-**3' (SEQ ID NO:44)**;

VH1BACKSFI15, 5'-CAT GCC ATG ACT CGC GGC CCA GCC GGC CAT GGC C(C/G)A GGT (C/G)(A/C)A (A/G)CT GCA G(C/G)A GTC (A/T)GG-**3' (SEQ ID NO:45)**;

FABNOTFOH, 5'-CCA CGA TTC TGC GGC CGC TGA AGA TTT GGG CTC AAC TTT CTT GTC GAC-**3' (SEQ ID NO:46)**;

FABNOTFOK 5'-CCA CGA TTC TGC GGC CGC TGA CTC TCC GCG GTT GAA GCT CTT TGT GAC-**3' (SEQ ID NO:47)**;

MVKBAAPA, 5'-CAC AGT GCA CTC GAC ATT GAG CTC ACC CAG TCT CCA-**3' (SEQ ID NO:48)**;

MVKBASFI, 5'-CAT GAC CAC GCG GCC CAG CCG GCC ATG GCC GAC ATT GAG CTC ACC CAG TCT CCA-**3' (SEQ ID NO:49)**;

VK3F2NOT, 5'-TTC TGC GGC CGC CCG TTT CAG CTC GAG CTT GGT CCC-**3' (SEQ ID NO:50)**.

Restriction sites are underlined.

Rescue of Phage and Phagemid particles - -

Please replace the material bridging page 160 (starting at line 26) through page 161 (line 7) with the following rewritten paragraph:

- - The phagemid vector, pHEN1 (fig. **[26] 26(a)**), is based upon pUC119 and contains restriction sites (SfiI and NotI) for cloning the fusion proteins. Here the transcription of antibody-g3p fusions is driven from the inducible lacZ promoter and the fusion protein targetted to the periplasm by means of the pelB leader. Phagemid was rescued with VCSM13 helper phage in 2xTY medium containing no glucose or IPTG: under these conditions there is sufficient expression of antibody-g3p. Fab and scFv fragments of NQ10.12.5 cloned into pHEN1 for display were shown to bind to phOx-BSA (but not BSA) by ELISA (Table 5) using the same criterion as above.- -

On page 179, please replace lines 10-15 with the following rewritten paragraph:

- -The construct fdphoAla166 (derived in example 11) was converted back to the wild type residue (arginine) at position 166 by in vitro mutagenesis (Amersham International) using the printer

APARG166:5' TAGCATTTGCGCGAGGTCACA 3' **(SEQ ID NO:51)**.

This construct with the wild type insert was called fdphoArg166.

On page 183, please replace lines 1-17, with the following rewritten paragraph:

- - Phage-enzyme or free alkaline phosphatase (83ng) mixed with vector phage were passed through filters with a nominal molecular weight limit of 300,000 daltons (Ultrafree-MC filters, Millipore). Figure 35 A again shows that the band of Mr, 115,000 is the major product reactive with anti-BAP antiserum. This and the other minor products reactive with anti-BAP are present in material retained by the ultrafiltration membrane. Analysis of retained and flow through fractions of phage preparations derived from KS272 demonstrates that different molecular species are being separated by the ultrafiltration membranes. Figure **[35b]** **35B** shows the protein of Mr 115,000 is retained by the filter whereas the putative degradation products of Mr 95,000 and 60,000 found in phage preparations derived from KS272 cells, are not retained.- -

Please replace the material bridging page 183 (starting at line 18) through page 184 (line 3) with the following rewritten paragraph:

- - In mixture of alkaline phosphatase and vector phage Figure **[35c-f]** **35B(c-f)**, free alkaline phosphatase (dimer size of 94,000 daltons) is detected in the flow through as a monomer band with Mr 47,000 on denaturing polyacrylamide gels (figure 35B), while the cross reactive molecule found in vector phage preparations (Mr 45,000) is in retained on the filter (figure 35B). This suggests that the cross reactive molecule is part of the phage particle and underlines the fact that the ultrafiltration membranes are effecting a separation. Thus the expected fusion band in this phage-enzyme is present in material retained on ultrafiltration membranes demonstrating that it is part of a larger structure as would be expected for viral bound enzyme.- -

Please replace the material bridging page 184 (starting at line 13) through page 185 (line 5) with the following rewritten paragraph:

- - Chaidaroglou et al, 1988 supra have shown that substituting alanine for arginine at the active site (residue 166) leads to a reduction in the rate of catalysis. Preparations of phage displaying alkaline phosphatase with this mutation derived from TG1 and KS272 show reduced specific activities of 380 and 1400 mol substrate converted/mol phage/min respectively. Enzyme activity was measured in the retained and flow-through fractions prepared by ultrafiltration, shown in **[figure 35]** **Figure 35(A) and Figure 35(B)**. The bulk of activity from phage-enzyme was retained on the filters whereas the majority of activity from free enzyme passes through. Therefore, the enzyme activity in these fusions behaved as would be expected for virally associated enzyme (not shown). Little or no catalytic activity is measured in preparations of vector phage from either TG1 or KS272 cells (Table 7), indication that the catalytic activities above are due to phage enzyme and not contamination with bacterial phosphatase. Addition of phage particles to soluble enzyme does not have a significant effect on activity (Table 7). - -

On page 189, please replace lines 9-15, with the following

- - VK-TERM-FOR

5' TGG AGA CTG GGT GAG CTC AAT GTC GGA GTG AGA ATA GAA AGG 3' **(SEQ ID NO:52)** (overlapping with VK2BACK [example 14])

and

CH1-TERM-BACK

5'AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA TCT AGC TGA TAA ACC GAT ACA ATT AAA GGC 3' **(SEQ ID NO:53)** (overlapping with HuIgG1-4 CH1-FOR)- -

Please replace the material bridging page 191 (starting at line 28) through page 192 (line 4) with the following rewritten paragraph:

- - Aliquots of the ligation reaction were transformed into competent TG1/pUC19gIII and plated on SOB medium containing ampicillin at 100μg/ml and kanamycin at 50μg/ml. Colonies

were screened for the presence of a deletion by PCR with primers gIIIFUBA and KSJ12 (CGGAATACCCAAAAGAACTGG)**(SEQ ID NO:54)**.- -

On page 192, please replace lines 5-16, with the following rewritten paragraph:

- - KSJ 12 anneals to gene VI which is immediately downstream of gIII in the phage genome, so distinguishing gIII on the helper phage from that resident on the plasmid. Three clones gave **[trucated]** **trunctated** PCR products corresponding to deletions of ca. 200, 400 and 800bp. These clones were called M13K07 gIII Δ Nos 1,2 and 3 respectively. No clones were isolated from the earlier Bal 31 time points, suggesting that these are in some way lethal to the host cell. Several clones were isolated from later time points, but none of these gave a PCR product, indicating that the deletion reaction had gone too far.- -

On page 193, please replace lines 5-18, with the following rewritten paragraph:

- - Only a minute fraction of the gIII protein on the M13K07-rescued material is present as an intact fusion **[fig 38]** **(Figs. 38A-38B)**. The fusion protein band is induced by IPTG, so is indisputably that synthesised by the phagemid. As expected, even when the lac promoter driving gIII fusion protein synthesis is fully induced (100μM IPTG), wild type gIII protein, at a lower copy number and driven from a far weaker promoter, predominates. This is in contrast to the pattern generated by the same clone rescued with M13K07 gIIIΔNo3, and the pattern generated by fd CAT2-scFv D1.3. In both of these latter cases, there is no competition with wild-type gIII and the fusion protein band is correspondingly stronger.- -

On page 195, please replace lines 12-25 with the following:

- - OLIGONUCLEOTIDES

VHBHD13APA : 5'- CAC AGT GCA CAG GTC CAA CTG CAG GAG AGC GGT-**3' (SEQ ID NO:55)**

VHFHD13 : 5'- CGG TGA CGA GGC TGC CTT GAC CCC-**3' (SEQ ID NO:56)**

HD13BLIN : 5'- GGG GTC AGG GCA GCC TCG TCA CCG-**3' (SEQ ID NO:57)**

HD13FLIN3 : 5'- TGG GCT CTG GGT CAT CTG GAT GTC CGA T-**3' (SEQ ID NO:58)**

VKBHD13 : 5'- GAC ATC CAG ATG ACC CAG AGC CCA-3' (SEQ ID NO:59)

VKFHD13NOT : 5'- GAG TCA TTC TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC -3' (SEQ ID NO:60)

MURD13SEQ : 5'- GAG GAG ATT TTC CCT GT-3' (SEQ ID NO:61)

HUMD13SEQ : 5'- TTG GAG CCT TAC CTG GC-3' (SEQ ID NO:62)

FDPCRFOR : 5'- TAG CCC CCT TAT TAG CGT TTG CCA-3' (SEQ ID NO:63)

FDPCRBAK : 5'- GCG ATG GGT GTT GTC ATT GTC GGC-3' (SEQ ID NO:64).- -

On page 199, please replace lines 20-24 with the following:

- -	TPB1	VH-HuH2-$(Gly_4-Ser)_3$-VK-HuK3	13 nM (SEQ ID NO:269)
	TPB2	VH-HuH1-$(Gly_4-Ser)_3$-VK-HuK4	180 Nm (SEQ ID NO:270)
	TPB3	VH-HuH2-$(Gly_4-Ser)_3$-VK-HuK4	(Unknown) (SEQ ID NO:271)
	TPB4	VH-HuH1-$(Gly_4-Ser)_3$-VK-HuK3	52 nM (SEQ ID NO:272) - -

Please replace the material bridging page 203 (starting at line 26) through page 204 (line 18) with the following rewritten paragraph:

- -The gene for the enzyme Staphylococcal nuclease (SNase) was amplified from M13 mp18 - SNase (Neuberger, M.S. et al Nature 312 604-608, 1984) by PCR using primers with internal ApaLI (5'- GGAATTCGTGCACAGAGTGCAACTTCAACTAAAAAATTAC-3')(SEQ ID NO:65) and NotI (5'-GGGATCCGCGGCCGCTTGACCTGAATCAGCGTTGTCTTCG-3') (SEQ ID NO:66) restriction sites, cloned into phage vector fd-CAT2 after digestion with ApaLI-NotI restriction enzymes and the nucleotide sequence of the SNase gene and junctions with gene III checked by DNA sequencing. The fd-tet-SNase phage was prepared from the supernatant of infected E.coli TG1 cultures by three rounds of PEG precipitation, and the fusion protein demonstrated by SDS-gel electrophoresis and Western blotting using rabbit anti-g3p antiserum (Prof. I. Rasched, Konstanz) and peroxidase-labelled goat anti-rabbit antibodies (Sigma) (Fig.41)

as described in example 27. As well as the fusion protein band (calculated Mr 59749, but runs at a higher position due to the aberrant g3p behaviour), a smaller (proteolytic ?) product is seen.- -

Please replace the material bridging page 205 (starting at line 12) through page 206 (line 9) with the following rewritten paragraph:

- - The protein CD4, a member of the immunoglobulin superfamily, is a cell surface receptor involved in MHC class II restricted immune recognition. It is also recognised by the protein gp120 derived from the human immunodeficiency virus (AIDS virus). The first two domains (named V1 and V2, residues 1-178) of the surface antigen CD4 were amplified from pUC13-T4 (gift from T. Simon) containing the human cDNA of CD4, by PCR using primers with internal ApaLI (5'-GGA ATT CGT GCA CAG AAG AAA GTG GTG CTG GGC AAA AAA GGG G-3') **(SEQ ID NO:67)** and NotI (5'-GGG ATC CGC GGC CGC AGC TAG CAC CAC GAT GTC TAT TTT GAA CTC-3') **(SEQ ID NO:68)** restriction sites. After digestion with these two enzymes, the PCR-product was cloned into fdCAT2, and the complete nucleotide sequence of the CD4-V1V2 DNA and junctions with gene III checked by dideoxy sequencing using oligonucleotides fd-seq1 (5'-GAA TTT TCT GTA TGA GG)**(SEQ ID NO:69)**, CD4-seq1 (5'-GAA GTT TCC TTG GTC CC-3')**(SEQ ID NO:70)** and CD4-seq2 (5'-ACT ACC AGG GGG GCT CT-3')**(SEQ ID NO:71)**. In the same way, a fd-CD4-V1 version was made, linking residues 1-107 to the N-terminus of gene III, using previously mentioned primers and oligonucleotide 5'-GGG ATC CGC GGC CGC GGT GTC AGA GTT GGC AGT CAA TCC GAA CAC-3' **(SEQ ID NO:72)** for amplification, PCR conditions and cloning were essentially as described in example 15 except that digestion was with ApaLI and NotI (used according to the manufacturers instructions).- -

Please replace the material bridging page 211 (starting at line 15) through page 212 (line 5) with the following rewritten paragraph:

- - After 4 rounds of mutation and selection, isolated clones were screened and in one or two rare examples strongly positive ELISA signals were obtained from phage antibodies derived from the mutation of each of fdCAT2scFvB18 and fdDOGKanscFvB18 in the ELISA. The

ELISA conditions were such that the parent phage fdCAT2scFvB18 only generated weak signals. These phage antibodies giving strongly positive ELISA signals were enriched in further rounds by a factor of roughly 2.5 per round. Forty phage antibodies giving strongly positive signals were sequenced and they each displayed single mutations in six different positions in the scFvB18 nucleotide sequences, five of which reside in the light chain. More than 70% of the mutations occurred at positions 724 and 725 changing the first glycine in the J segment of the light chain (framework 4) to serine (in 21 cases) or aspartate (in 3 cases). The mutations found are shown in Table 9. The sequence of scFvB18 is shown in Figure 44**(i) through 44(ii)**.- -

Please replace the material bridging page 218 (starting at line 13) through page 219 (line 2) with the following rewritten paragraph:

- - The overall strategy for the PCR assembly is shown in fig.47 and is described in more detail below. For Fab assembly, the VH-CH1 and VK-CK or V lambda-C lambda light chains are amplified from first strand cDNA and gel purified. Heavy and light chain DNA are then combined together with linker DNA and flanking oligonucleotides in a new PCR reaction. This results in a full length Fab construct since the 5' end of the linker DNA is complementary to the 3' end of the CH1 domain and the 3' end of the linker is complementary to the 5' end of the light chain domain. The linker DNA contains terminal residues of the human CH1 domain, the bacterial leader sequence (pelB) for the light chain and the initial residues of the VK or V lambda light chain**[(fig.2)]**. Finally, after gel purification, the Fab construct is reamplified with flanking oligonucleotides containing restriction sites for cloning.- -

Please replace the material bridging page 227 (starting at line 20) through page 228 (line 25) with the following rewritten paragraph:

- - The human hybridoma Fog-B has been previously described (N.C. Hughes-Jones et al Biochem, J. 268 135 (1990). It produces an IgG-1/lambda antibody which binds the Rh-D antigen. RNA was prepared from 10^7 hybridoma cells using a modified method of Cathala (as described in example 14) and 1st strand cDNA synthesized using specific immunoglobulin heavy and light chain primers (HuVH1FOR [example 40] and HuCλ FOR (5'-GGA ATT CTT

ATG AAG ATT CTG TAG GGG CCA C-3')**(SEQ ID NO:73)**) as described in example 14. The VH gene was subsequently amplified from an aliquot of the 1st strand cDNA using HuVH4aBACK and HuVH1FOR. The Vλ gene was amplified using a Vλ primer specific for Fog-B (VλFog-B[,]: 5'-AAC CAG CCA TGG CC AGT CTG TGT TGA CGC AGC C-3')**(SEQ ID NO:74)**. The PCR conditions were as described in example 40. The PCR products were analyzed by running 5μl on a 2% agarose gel. The remainder was extracted twice with ether, twice with phenol/chloroform, ethanol precipitated and resuspended in 50μl of H_2O. The amplified VH DNA was digested with Pst1 and BstEII, and the amplified Vλ-Cλ DNA with Ncol and EcoR1. The fragments were purified on a 2% agarose gel, extracted using Geneclean, and sequentially ligated into the soluble expression vector pJM-1 Fab D1.3 (Fig 48**(i)**). Clones containing the correct insert were initially identified by restriction analysis and verified by assay of expressed soluble Fab (see example 23 for induction conditions). The Fog-B Fab cassette was amplified from pJM-1 by PCR using HuVH4BACK-Sfi and Hu Cλ-Not, digested with the appropriate restriction enzymes and ligated into pHEN1. Clones containing the correct insert were identified initially by restriction analysis and subsequently by assay (see example 25 for induction conditions).- -

Please replace the material bridging page 248 (starting at line 19) through page 249 (line 17) with the following rewritten paragraph:

- -The oligonucleotides mutL91,92, was prepared too randomise phenylalanine at position 91 (L91) and tryptophan at position 92 (L92) of the light chain. The oligonucleotides mutL32, was prepared to randomise tyrosine at light chain position 32 (L32) and the oligonucleotides mutH101 was prepared to randomise tyrosine at position 101 of the heavy chain (H101).

mutL91,92:

5' CGT CCG AGG AGT ACT NNN NNN ATG TTG ACA GTA ATA 3' **(SEQ ID NO:75)**

mutL32:

5' CTG ATA CCA TGC TAA NNN ATT GTG ATT ATT CCC 3' **(SEQ ID NO:76)**

mutH101:

5' CCA GTA GTC AAG CCT NNN ATC TCT CTC TCT GGC 3' **(SEQ ID NO:77)**

(N represents a random insertion of equal amounts of A,C,G or T) in vitro mutagenesis of the phagemid vector, pCAT3scFvD1.3 (example 17) with the oligonucleotide mutL91,92 was carried out using an in vitro mutagenesis kit (Amersham). The resultant DNA was transformed by electroporation into TG1 cells using a Bio-Rad electroportor. 78,000 clones were obtained and these were scraped into 15mls of 2xTY/20% glycerol. This pool was called D1.3L91L92. Single stranded DNA was prepared by rescue with M13K07 as described in Sambrook et al, 1989 supra, and sequenced with the primer FDTSEQ1, using a Sequenase sequencing kit (United States Biochemical Corporation).- -

On page 252, please replace lines 22-29, with the following rewritten paragraph:

- - A dilution series was made on 10 clones which were analysed by ELISA in 6 of these clones the profile of binding to HEL was the same as the original clone (pCAT3SCFvD1.3) while the signal with TEL was increased (see figure 50**(i)** clone B1). In the remaining 4 clones, the increased signal with TEL was accompanied by a decrease in signal on HEL (see figure 50 clone A4).- -

Please replace the material bridging page 258 (starting at line 22) through page 259 (line 5) with the following rewritten paragraph:

- - After 1 hour of incubation with mixing at room temperature, magnetic beads were recovered using a Dynal MPC-E magnetic desperation device. They were then washed in PBS containing 0.5% Tween 20, (3x10 minutes, 2x1 hour, 2x 10 minutes) and phage eluted by 5 minutes incubation in 50µl PBS containing 10mM dithiothreitol. The eluate was used to infect TG1 cells and the resulting colonies probed with the oligo NQ11CDR3 (5' AAACCAGGCCCCGTAATCATAGCC 3') **(SEQ ID NO:80)** derived from CDR3 of the NQ11 antibody (This hybridises to pAbNO11 but not pAb D1.3).- -

Replace Table 10(i) on page 280 with the table on the following page:

Table 10(i) Oligonucleotide primers used for PCR of human immunoglobulin genes

Oligo Name	Sequence
Human VH Back Primers	
HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3' **(SEQ ID NO:81)**
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3' **(SEQ ID NO:82)**
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3' **(SEQ ID NO:83)**
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3' **(SEQ ID NO:84)**
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3' **(SEQ ID NO:85)**
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3' **(SEQ ID NO:86)**
HuVH1aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3' **(SEQ ID NO:87)**
HuVH2aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3' **(SEQ ID NO:88)**
HuVH3aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3' **(SEQ ID NO:89)**
HuVH4aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3' **(SEQ ID NO:90)**
HuVH5aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3' **(SEQ ID NO:91)**
HuVH6aBACKSfi	5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3' **(SEQ ID NO:92)**
Human JH Forward Primers	
HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3' **(SEQ ID NO:93)**
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3' **(SEQ ID NO:94)**
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3' **(SEQ ID NO:95)**
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3' **(SEQ ID NO:96)**
Human Heavy Chain Constant Region Primers	

Replace Table 10(ii) on page 281 with the table on the following page:

Table 10(ii)

HuIgG1-4CH1FOR	5'-GTC CAC CTT GGT GTT GCT GGG CTT-3' **(SEQ ID NO:97)**
HuIgMFOR	5'-TGG AAG AGG CAC GTT CTT TTC TTT-3' **(SEQ ID NO:98)**

Human Vκ Back Primers

HuVκ1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3' **(SEQ ID NO:99)**
HuVκ2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3' **(SEQ ID NO:100)**
HuVκ3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3' **(SEQ ID NO:101)**
HuVκ4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3' **(SEQ ID NO:102)**
HuVκ5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3' **(SEQ ID NO:103)**
HuVκ6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3' **(SEQ ID NO:104)**

Human Jκ Forward Primers

HuJκ1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3' **(SEQ ID NO:105)**
HuJκ2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3' **(SEQ ID NO:106)**
HuJκ3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3' **(SEQ ID NO:107)**
HuJκ4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3' **(SEQ ID NO:108)**
HuJκ5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3' **(SEQ ID NO:109)**
HuJκ1BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3' **(SEQ ID NO:110)**
HuJκ2BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAG CTT GGT CCC-3' **(SEQ ID NO:111)**
HuJκ3BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3' **(SEQ ID NO:112)**
HuJκ4BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3' **(SEQ ID NO:113)**
HuJκ5BACKNot	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3' **(SEQ ID NO:114)**

Human κ Constant Region Primers

Please replace Table 10(iii) on page 282 with the table on the next page:

Table 10(iii)

HuCκFOR	5'-AGA CTC TCC CCT GTT GAA GCT CTT-3' **(SEQ ID NO:115)**
HuCκFORNot1	5'-GAG TCA TTC TCG ACT TGC GGC CGC TTA TTA AGA CTC TCC CCT GTT GAA GCT CTT-3' **(SEQ ID NO:116)**
HuCκFORNot2	5'-GAG TCA TTC TCG ACT TGC GGC CGC AGA CTC TCC CCT GTT GAA GCT CTT-3' **(SEQ ID NO:117)**

Human λ Back Primers

HuVλ1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3' **(SEQ ID NO:118)**
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3' **(SEQ ID NO:119)**
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3' **(SEQ ID NO:120)**
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3' **(SEQ ID NO:121)**
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3' **(SEQ ID NO:122)**
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3' **(SEQ ID NO:123)**
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3' **(SEQ ID NO:124)**

Human λ Forward Primers

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3' **(SEQ ID NO:125)**
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3' **(SEQ ID NO:126)**
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3' **(SEQ ID NO:127)**

HuJλFORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3' **(SEQ ID NO:128)**
HuJλ2-3FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3' **(SEQ ID NO:129)**
HuJλ4-5FORNOT	5'-GAG TCA TTC TCG ACT TGC GGC CGC ACY TAA AAC GGT GAG CTG GGT CCC-3' **(SEQ ID NO:130)**

Human λ Constant Region Primers

Replace Table 10(iv) from page 283 with the table on the following page:

Table 10(iv)

HuCλFOR	5'-TGA AGA TTC TGT AGG GGC CAC TGT CTT-3' **(SEQ ID NO:131)**
HuCλFORNot1	5'-GAG TCA TTC TCG ACT TGC GGC CGC TTA TTA TGA AGA TTC TGT AGG GGC CAC TGT CTT-3' **(SEQ ID NO:13**
HuCλFORNot2	5'-GAG TCA TTC TCG ACT TGC GGC CGC TGC AGA TTC TGT AGG GGC TGT CTT-3' **(SEQ ID NO:133)**

Linker oligos

Reverse JH for scFv linker

RHuJH1-2	5'-GCA CCC TGG TCA CCG TCT CCT CAG GTG G-3' **(SEQ ID NO:134)**
RHuJH3	5'-GGA CAA TGG TCA CCG TCT CTT CAG GTG G-3' **(SEQ ID NO:135)**
RHuJH4-5	5'-GAA CCC TGG TCA CCG TCT CCT CAG GTG G-3' **(SEQ ID NO:136)**
RHuJH6	5'-GGA CCA CGG TCA CCG TCT CCT CAG GTG C-3' **(SEQ ID NO:137)**

Reverse IgG1-4CH1 primer for Fab linker

RhuIgG1-4CH1FOR	5'-AAG CCC AGC AAC ACC AAG GTG GAC-3' **(SEQ ID NO:138)**

Reverse Vκ for scFv linker

RhuVκ1aBACKFv	5'-GGA GAC TGG GTC ATC TGG ATG TCC GAT CCG CC-3' **(SEQ ID NO:139)**
RhuVκ2aBACKFv	5'-GGA GAC TGA GTC ATC ACA ACA TCC GAT CCG CC-3' **(SEQ ID NO:140)**
RhuVκ3aBACKFv	5'-GGA GAC TGC GTC AAC ACA ATT TCC GAT CCG CC-3' **(SEQ ID NO:141)**
RhuVκ4aBACKFv	5'-GGA GAC TGG GTC ATC ACG ATG TCC GAT CCG CC-3' **(SEQ ID NO:142)**
RhuVκ5aBACKFv	5'-GGA GAC TGC GTG AGT GTC GTT TCC GAT CCG CC-3' **(SEQ ID NO:143)**
RhuVκ6aBACKFv	5'-GGA GAC TGA GTC AGC ACA ATT TCC GAT CCG CC-3' **(SEQ ID NO:144)**

Reverse Vκ for Fab linker

Please replace Table 10(v) from pages 284 and 285 with the following table:

Table 10(v)

RHuVκ1aBACKFab	5'-GGA GAC TGG GTC ATC TGG ATG TCG GCC ATC GCT GG-3' **(SEQ ID NO:145)**
RHuVκ2aBACKFab	5'-GGA GAC TGC GTC ATC ACA ACA TCG GCC ATC GCT GG-3' **(SEQ ID NO:146)**
RHuVκ3aBACKFab	5'-GGA GAC TGC GTC AAC ACA ATT TCG GCC ATC GCT GG-3' **(SEQ ID NO:147)**
RHuVκ4aBACKFab	5'-GGA GAC TGG GTC ATC ACG ATG TCG GCC ATC GCT GG-3' **(SEQ ID NO:148)**
RHuVκ5aBACKFab	5'-GGA GAC TGC GTG AGT GTC GTT TCG GCC ATC GCT GG-3' **(SEQ ID NO:149)**
RHuVκ6aBACKFab	5'-GGA GAC TGC GTC AGC ACA ATT TCG GCC ATC GCT GG-3' **(SEQ ID NO:150)**

Reverse Vλ for svFv linker

RHuVλBACK1Fv	5'-GGC GGC TGC GTC AAC ACA GAC TGC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:151)**
RHuVλBACK2Fv	5'-GCA GGC TGA GTC AGA GCA GAC TGC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:152)**
RHuVλBACK3aFv	5'-GGT GGC TGA GTC AGC ACA TAG GAC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:153)**
RHuVλBACK3bFv	5'-GGG TCC TGA GTC AGC TCA GAA GAC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:154)**
RHuVλBACK4Fv	5'-GGC GGT TGA GTC AGT ATA ACG TGC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:155)**
RHuVλBACK5Fv	5'-GAC GGC TGA GTC AGC ACA GAC TGC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:156)**
RHuVλBACK6Fv	5'-TGG GGC TGA GTC AGC ATA AAA TTC GAT CCG CCA CCG CCA GAG-3' **(SEQ ID NO:157)**

Reverse Vλ for Fab linker

RHuVλBACK1Fab	5'-GGC GGC TGC GTC AAC ACA GAC TGG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:158)**
RHuVλBACK2Fab	5'-GCA GGC TGA GTC AGA GCA GAC TGG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:159)**
RHuVλBACK3aFab	5'-GGT GGC TGA GTC AGC ACA TAG GAG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:160)**
RHuVλBACK3bFab	5'-GGG TCC TGA GTC AGC TCA GAA GAG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:161)**
RHuVλBACK4Fab	5'-GGC GGT TGA GTC AGT ATA ACG TGG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:162)**
RHuVλBACK5Fab	5'-GAC GGC TGA GTC AGC ACA GAC TGG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:163)**
RHuVλBACK6Fab	5'-TGG GGC TGA GTC AGC ATA AAA TTG GCC ATC GCT GGT TGG GCA-3' **(SEQ ID NO:164)**

Please replace Table 11 on page 286 with the table on the next page:

Table 11. Deduced protein sequences of heavy and light chains selected from unimmunized library

Oxazolone binder

HEAVY CHAIN
VH15.4 QVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGIS WVRQAPGQGLEWMG WISAYNGNTKYAQKLQG RVTMITDTSTSTAYMELRSLRSDDTAVYYCVR LLPKRTATLH YYIDVWGKGT (SEQ ID NO:165)

LIGHT CHAIN
VL15.4 NNYVS WYQHLPGTAPNLLIY DNNKRPS GIPDRFSGSKSGTSATLGITGLQTGDEADYYC GIWDGR (SEQ ID NO:166)

BSA Binders

HEAVY CHAINS
VH3.5 QVQLVQSGGGVVQPGRSLRLSCAASGFTFS SYGMH WVRQAPGKGLEWVA VISYDGSNKYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK TGYSSGWGY FDYWGQGT (SEQ ID NO:167)

LIGHT CHAINS
VL3.5 SSELTQDPAVSVALGQTVRITC QGDSLRSYYAS WYQQKPGQAPVLVIY GKNNRPS GIPDRFSGSSSGNTASLTITGAQAEDEADYYC NSRDSSGNH VVFGG (SEQ ID NO:168)

Lysozyme binders:

HEAVY CHAINS
VH10.1 SLTCSVSGDSIS SGGYS WIRQPSGKGLEWIG SVHHSGPTYYNPSLKS RVTMSVDTSKNQFSLKLKSVTAADTAMYFCAR EGGSTWRSLYKH YYMDVWGK (SEQ ID NO:169)
VH14.1 QVQLQESGPGLVKPSETLSLVCTVSGGSLS FSYWG WIRQPPGKGLEWIG YISHRGTDYNSSLQS RVTISADTSKNQFSLKLSSVTAADTAVYYCAR SFSNSFFFGY WGQGT (SEQ ID NO:170)
VH13.1 QVQLVQSGAEVKKPGQSLMISCQGSGYSFS NYWIG WVRQMPGKGLEWMG IIYPGDSDTRYSPSFQG QVTISADKSISTAYLHWSSLKASDTALYYCAR LVGGTPAY WGQGT (SEQ ID NO:171)
VH16.1 QVQLVQSGAEVKKPGQSLRISCKGAGYSFS TYWIG WVRQMPGKGLEWMG IIYPDDSDTRYSPSFEG QVTISVDKSITTAYLHWSSLKA (SEQ ID NO:172)

LIGHT CHAINS
VK10.1 EIVLTQSPSSLSASVGDRVTITC RASQSISNYLN WYQQKPGKAPKLLIY AASTLQS GVPSRFSGSGSGTDFTLTINSLQPEDFATYYC QQTIISFP LTFGGG (SEQ ID NO:173)
VL14.1 SSELTQDPAVSVAFGQTVRITC QGDSLRSSYAS WYQQKPGQAPLLVIY GENSRPS GIPDRFSGSSSGNTASLTITGAQAEDEADYYC NSRDSRGTHL EVFGG (SEQ ID NO:174)
VL13.1 HVILTQPASVSGSPGQSITISC TGSSRDVGGYNYVS WYQHHPGKAPKLLIS EVTNRPS GVSNRFSGSKSGNTASLTISGLQAEDEADYFC ASYTSSKT YVFGG (SEQ ID NO:175)
VL16.1 QSALTQPASVSGSPGQSITISC SGSSSDIGRYDYVS WYQHYPDKAPKLLIY EVKHRPS GISHRFSASKSGNTASLTISELQPGDEADYYC ASYT (SEQ ID NO:176)